

# ISOTOPIC GAS ANALYSIS FOR BIOCHEMISTS

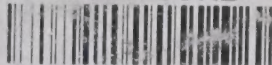
R. F. Glascock

GX3,B1

N54

5751

CFTRI-MYSORE



5751

Isotopic gas ana



TLMS







ISOTOPIC GAS ANALYSIS FOR BIOCHEMISTS



# ISOTOPIC GAS ANALYSIS FOR BIOCHEMISTS

R. F. GLASCOCK

*Head of the Isotope Section  
The National Institute for Research in Dairying  
University of Reading  
Reading, England*



ACADEMIC PRESS INC., Publishers

New York, 1954

5751

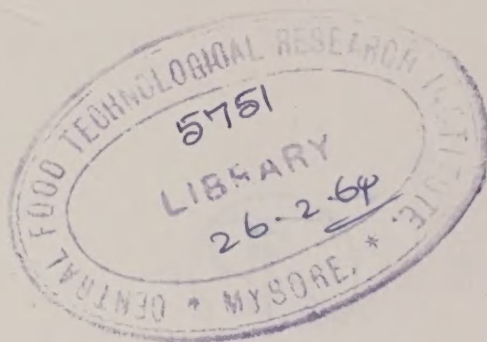
at 26.2.64

Copyright, 1954, by  
ACADEMIC PRESS INC.  
125 East 23rd Street  
New York 10, N. Y.

*All Rights Reserved*

No part of this book may be reproduced in  
any form, by photostat, microfilm, or by any  
other means, without written permission from  
the publishers.

*Library of Congress Catalog Card Number: 54-6348*

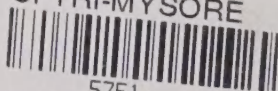


GX3,B1

N54

PRINTED IN THE UNITED STATES OF AMERICA

CFTRI-MYSORE



5751  
Isotopic gas ana.

## PREFACE

Papers on the gas phase measurement of carbon and hydrogen isotopes have now become sufficiently numerous in the literature, and the methods themselves have aroused sufficient interest among biochemists, for a laboratory manual on the subject to appear worth-while. The particular advantages, especially in biochemical work, are discussed in the introduction. The author himself is responsible for a laboratory in which gas phase counting is the chief method of measuring these isotopes in the course of a fairly extensive program of biochemical and physiological research; he therefore hopes that the prominence given in this book to those methods of which he has first-hand experience will be thought justified. These are the ones described as carried out by means of *permanent vacuum-line technique*. Information on all others has been taken from the literature, augmented in a very few instances by private communications. It is hoped that any method described can be applied directly without its being necessary to refer back to the original paper. Full acknowledgment is given for all methods quoted but, unless the contrary is explicitly stated, it is to be understood that the author has not tested them himself but depends for criteria of reliability on the published data.

Acknowledgment is gladly made to all those whose permission to copy published diagrams has been obtained. The liberty of slightly simplifying diagrams has in some cases been taken, and it is hoped that the reason for so doing will be apparent from the context.

The author is also happy to acknowledge the patient and understanding cooperation of Mrs. D. E. Jones, who is responsible for all the drawings. The necessity of asking her to copy diagrams straight from the original papers has made it possible to use the same conventions in every drawing for commonly used items



such as stopcocks and standard joints. In the diagrams of the permanent vacuum line used by the author, however, the same conventions and notation have been used throughout the book, all stopcocks being shown in the open position for the sake of clarity. It is also to be noted that the numbering and notation of the master figure, Fig. 1, have been repeated in all other diagrams showing various sections of that apparatus.

While the author himself is alone responsible for any errors, thanks are gladly offered to all those whose valuable suggestions have helped him in compiling this book, and in particular to Dr. D. Taylor of the Atomic Energy Research Establishment, Harwell (Ionisation Chambers), Dr. D. H. Tomlin of the Physics Department, Reading University (Mass Spectrometry), and Mr. N. B. Balaam of 20th Century Electronics Ltd. (Counters); also to Dr. S. J. Folley, F.R.S., for his encouragement and advice; and to Mr. W. G. Duncombe and Mr. B. W. E. Peaple for help in the development of several of the methods described and for help in correcting the manuscript.

R. F. GLASCOCK  
Reading, England

*June, 1954*

## TABLE OF CONTENTS

PREFACE . . . . .	v
I. Introduction, Outline of Radioactive Assay Methods . . . . .	1
References . . . . .	9
II. High-Vacuum Apparatus . . . . .	10
General Requirements . . . . .	10
Gas Manipulation in a Vacuum . . . . .	26
Gas Measurements . . . . .	26
Additional Apparatus . . . . .	43
References . . . . .	47
III. Gas Phase Assay of Carbon-14 . . . . .	48
Discharge Tubes . . . . .	48
Elementary Theory. . . . .	48
Ionization Chambers . . . . .	50
Measurement of $C^{14}O_2$ in Ionization Chambers . . . . .	53
Counting Tubes. . . . .	58
Proportional Counting of Carbon Dioxide . . . . .	66
Using Methane-Carbon Dioxide Mixtures . . . . .	66
Using Carbon Dioxide Alone in the Proportional Counter . . . . .	68
Measurement of $C^{14}$ as Acetylene for Radiocarbon Dating . . . . .	71
References . . . . .	73
IV. Gas Phase Assay of Carbon-14 (Continued) . . . . .	75
Geiger Counting of Carbon Dioxide . . . . .	75
References . . . . .	86
V. Combustion of Labeled Compounds . . . . .	87
Wet Combustion Methods . . . . .	88
Methods Using Dry Combustion . . . . .	101
The Production of Free Carbon Dioxide and Water by Combustion . . . . .	102
The Production of Large Samples of Combustion Water. . . . .	111
References . . . . .	115
VI. Combustion of Samples Containing Deuterium, Tritium, $C^{13}$ , and $C^{14}$ , Using Permanent Vacuum Line . . . . .	116
Apparatus . . . . .	117
References . . . . .	133
VII. The Determination of Deuterium . . . . .	134
Determination of Deuterium by the Falling Drop Method . . . . .	135

The Mass Spectrometric Determination of Deuterium, Using Hydrogen . . . . .	146
The Reduction of Water to Hydrogen . . . . .	147
The Mass Spectrometric Determination of Deuterium Using Hydrocarbons . . . . .	152
References . . . . .	156
VIII. The Determination of Tritium . . . . .	157
Water Vapor as a Medium for Tritium Assay . . . . .	160
Hydrogen Gas as a Medium for Tritium Assay . . . . .	163
Ionization Chamber Methods . . . . .	164
Counting of Hydrogen . . . . .	168
Counting of Tritio-Hydrocarbons . . . . .	171
Methane . . . . .	171
Hydrogen-Methane Mixtures . . . . .	178
References . . . . .	179
IX. The Determination of Tritium (Continued) . . . . .	180
Assay as Tritio-Butane Using Permanent Vacuum-Line Technique . . . . .	180
Preparation of Tritio-Butane from Water . . . . .	182
Butane-Filled Counters . . . . .	190
Reproducibility of Assays . . . . .	192
Wet Combustion of High-Specific-Activity Tritium Compounds . . . . .	193
References . . . . .	194
X. The Preparation of Heavy Nitrogen Samples for Mass Spectrometric Analysis . . . . .	195
Micro-Kjeldahl Method (Rittenberg) . . . . .	195
Micro-Dumas Method (Holt and Hughes) . . . . .	198
References . . . . .	201
XI. The Preparation of Tritium-Labeled Compounds . . . . .	202
General Precautions . . . . .	202
The Preparation of Tritiated Water . . . . .	203
Preparation of Tritium-Labeled Acetic Acid . . . . .	208
The Preparation of Tritiated Organic Compounds by Exchange Without Catalyst . . . . .	210
Using Metal Catalysts . . . . .	211
Catalytic Hydrogenation . . . . .	216
A Microhydrogenation Technique . . . . .	221
References . . . . .	228
XII. The Study of <i>In Vitro</i> Oxidation . . . . .	230
Permanent-Vacuum-Line Gas Counting Method . . . . .	230
References . . . . .	236
AUTHOR INDEX . . . . .	239
SUBJECT INDEX . . . . .	242

## CHAPTER I

### INTRODUCTION

#### OUTLINE OF RADIOACTIVE ASSAY METHODS

##### *End-Window Counters*

The simplest and most commonly used method of measuring the activity of radioactive materials depends on the use of an end-window counter, beneath which the solid sample is placed. Even with samples emitting high-energy radiations, the geometry of this arrangement permits only a relatively small proportion of the radiations to enter the sensitive zone of the counter. If they are of low energy, such as those emitted by  $C^{14}$  (maximum energy 0.15 Mev) there is a further considerable loss of efficiency by absorption at the window of the counter and by self-absorption in the sample. Window absorption is reduced to a minimum by making it of very thin mica, usually less than 2 mg./cm.<sup>2</sup>, or by the use of an internal gas-flow counter, which eliminates it altogether and much increases geometrical efficiency.

##### *Self-absorption*

The problem of self-absorption by weak emitters such as  $C^{14}$  can be dealt with in a number of ways. One method is to spread the sample in a thin layer so that self-absorption is negligible. This means that a relatively large area must be covered if reasonable counting rates are to be obtained. Entenman *et al.* (1) have described a method for counting very thin samples of fatty acids by deposition of 5–30-mg. samples from solution on a lens paper of total area 15 cm.<sup>2</sup>. For such a method to be applicable, however, the substance used must be one that can be deposited from solution in a uniform layer (which is not possible for all substances),

and the counter must be designed so that most of the radiations from such a sample disk will enter the sensitive zone.

Another method of counting is to use a known thickness and correct the observed activity to zero thickness, i.e., to the value that would have been observed if no self-absorption had occurred. For this purpose, a self-absorption curve must be constructed by plotting counting rate against thickness for a sample of known specific activity. For accurate work, the self-absorption curve for every class of compound counted must be obtained because they will not all be identical, owing to backscattering (see below).

The commonest method of dealing with the problem of self-absorption, however, is to count at infinite thickness at which, for any particular chemical compound, the observed activity is dependent only on the specific activity of the sample. At infinite thickness, the radiations from the lowest layers are completely absorbed before reaching the surface and therefore never enter the counter; it corresponds with the horizontal portion of the self-absorption curve where the counting rate is independent of thickness. Its actual minimum value depends on the energy of the radiations emitted and is 20 mg./cm.<sup>2</sup> for C<sup>14</sup> (and less than 1 mg./cm.<sup>2</sup> for tritium).

#### *Determination of C<sup>14</sup>*

The specific activity of the carbon contained in the sample is the quantity usually sought, and, for a given chemical compound, this may be found by calculating the activity that the carbon would have if isolated and counted at infinite thickness; this activity is then multiplied by an empirical factor to give specific activity. Many workers omit the second part of the calculation since a valid comparison of carbon specific activities may be made by comparing activities at infinite thickness. This is given by

$$A_c = \frac{100A_s}{C}$$

where  $A_c$  is the activity of the carbon,  $A_s$  is the observed activity of the sample, and  $C$  is the percentage of carbon contained in it.

For a comparison of the specific activity of carbon in com-



pounds of markedly different chemical composition, however, the effect of backscattering must be taken into consideration. More radiations than would be expected from a consideration only of the specific activity of the carbon and absorption in the sample are emitted from thick samples, particularly those containing heavy elements. This is due to reflection back to the surface of radiations originally directed away from the counter. The increase in counting rate due to this backscattering may be by as much as a factor of 1.3 for barium carbonate or by as little as a factor of 1.04 for substances containing only light elements (2). The formula for calculating the activity of the carbon in the sample must therefore be modified to

$$A_c = \frac{100A_s}{BC}$$

where  $B$  is the backscattering factor. Unfortunately there is not universal agreement as to the value of backscattering factors, and some workers think it necessary to evaluate them for themselves for their own counters and on their own equipment.

### *Nature of the Sample*

An additional difficulty inherent in solid counting of organic samples is nonuniformity of surface. Some substances are much more easily spread in uniform layers than others, and truly comparable results obviously cannot be obtained unless each sample counted has the same effective surface area. An additional difficulty may arise from the necessity of counting substances which are not chemically pure and whose carbon content is not, therefore, accurately known. An example of such a class of compounds is the mixture of fatty acids extracted from tissue slices in studies on fat synthesis. Many workers therefore prefer to convert all their samples to a common chemical form; this obviates the necessity of calculating the specific activity of the carbon and of applying the backscattering correction with its possible attendant errors. At the same time it ensures a reasonably uniform surface which is the same for each sample. The compound usually chosen is barium carbonate obtained by combustion, absorption of the

carbon dioxide in barium hydroxide solution, and filtration, drying, and plating of the precipitate.

### *Gas Phase Measurement of Radioactivity*

It is particularly to be noted that organic samples are also reduced to a common chemical form by conversion to carbon dioxide; and the introduction of it into instruments designed for gas phase assay provides a reliable means of directly comparing carbon specific activities. Once the apparatus has been built, it is considerably easier to introduce the carbon dioxide into a counter or ionization chamber than to convert to barium carbonate and plate it ready for solid counting. Furthermore, this procedure results in an increased instead of a diminished efficiency.

### *Relative Sample Size for Solid and Gas Counting*

Apart from those substances which are suitable for spreading in very thin layers over large surfaces, it is usually inconvenient, especially for counting at infinite thickness, if less than about 75 mg. sample are available. It is true that less than this can be spread on small disks 1 cm.<sup>2</sup> in area but, for the purpose of avoiding inaccuracies due to edge effects, most workers prefer disks of 2 or even 3 cm.<sup>2</sup> area which require 40 and 60 mg., respectively, of C<sup>14</sup> compounds for counting at infinite thickness. Thus, allowing for transfer losses, the 75 mg. quoted is not usually excessive. This is to be compared with the 5–10 mg. which is sufficient for many methods of gas phase assay. Thus, in a method described by the present author (Chapter VI), three isotopes (C<sup>13</sup>, C<sup>14</sup>, and tritium) can be determined in the products of combustion of a single 10-mg. sample. This is an important advantage since it may often be difficult to isolate more than this in biochemical experiments, and the addition of carrier, even if possible, may reduce the observed activity to an inconveniently low level. It is true that combustion and conversion to barium carbonate, already referred to as a procedure designed to eliminate the errors arising from the counting of chemically different compounds, brings about an effective increase in sample size; thus glucose yields 6.6 times its weight of barium carbonate. But this increase in weight

is, of course, accompanied by a proportional drop in specific activity.

TABLE 1  
COMPARISON OF  $C^{14}$  SOLID COUNTING AT INFINITE THICKNESS  
AND GAS COUNTING OF  $C^{14}O_2$

	I	II	III	IV	V	VI	VII
	Activity of 20 mg. $CO_2$ from on 1-cm. <sup>2</sup> combus- disk (counts/ min.)	Volume of 20 mg. (std. ml.)	Specific activity of $CO_2$ (counts/ min./ ml.)	Total activity of V ml. (counts/ min.)	Ratio $Z/X =$ gain in effi- ciency	Activity at $\infty$ thick- ness = $100A_s/$ BC (counts/ min.) (N)	Ratio N/Y
Substance	(X)	(V)	(Y)	(Z)			
Ca salts of mixed fatty acids containing $C^{14}$	2,439	24.6	4,150	102,000	42	3,620	0.87
$C^{14}$ -glucose	120	15.0	450	6,750	56	288	0.64
$BaC^{14}O_3$	26	2.28	320	730	28	329	1.03
$BaC^{14}O_3$	2,204	2.28	29,300	66,800	30	28,000	0.96

### Relative Efficiency of End-Window and Gas Counting

Gas counting is about 50 times more efficient than solid counting at infinite thickness using a counter fitted with a window of about 2 mg./cm.<sup>2</sup> Some figures accumulated in the author's laboratory (3), illustrating the differences between end-window and gas phase counting of some organic samples are given in Table 1. In column I is given the observed activity of each sample when counted on a 1 cm.<sup>2</sup> disk at infinite thickness (20 mg./cm.<sup>2</sup>). In column II is given the volume of carbon dioxide obtained from that 20 mg. by combustion or decomposition, and, in column III, its specific activity. Thus the product of the figures in these two columns gives the total observable activity in the  $CO_2$  obtainable from the 20 mg. combusted (the gas counter used held 25 standard ml. at its operating pressure), and is shown in column IV. This activity

is, in all cases, very much greater than the activity at infinite thickness, and the factor by which it is greater is shown in column V. It will be seen that the gain in efficiency is as much as 56 (for glucose).

Although this table is not the result of a rigorous comparative study of the two methods, it gives some indication of the reliability of the calculation of carbon specific activity from known carbon content, published backscattering factor, and observed activity at infinite thickness. The calculated infinite-thickness activity of the carbon in the samples is shown in column VI (the carbon content of the fatty acid calcium salts being obtained from a microanalysis). If it is a true measure of the specific activity of the carbon, then for all samples it should bear the same ratio to the specific activity of the carbon dioxide shown in column III. This ratio is shown in the last column and will be seen to vary between 0.64 for glucose and 1.03 for barium carbonate. The agreement for the ratio for the two barium carbonate samples is quite good and it would thus appear, apart from considerations of relative efficiency, that more reliable results are obtained if the compounds to be assayed are always reduced to the same chemical form. This is in agreement with the finding of other workers; for example, Abrams and Clark (4) who say they have been able to get  $C^{14}$  counts to agree within 5 per cent only by completely combusting the sample and counting as an infinitely thick layer of barium carbonate. In attempting to count thin samples of uncombusted compounds, they observed inaccuracies as high as 30 per cent. As already noted, however, it is easier to introduce carbon dioxide into a gas phase counter than to convert it to barium carbonate for solid counting.

### *Gas-flow Counters*

These counters are not factory-filled but are constructed so that the filling gas continually passes through them at a pressure slightly in excess of atmospheric, thus preventing the entry of air. They may be operated in the Geiger region by using helium containing a suitable quenching vapor (5, 6) or in the proportional region using methane (7, 8). The sample to be counted is intro-



duced into the body of the counter by means of a suitable mechanical device, the radiations from it then passing into the sensitive zone without interference by a window. There is also a very favorable geometry, and the resultant efficiency may be as high as 50 per cent under conditions of zero self-absorption. This is about ten times higher than is possible with an end-window counter. The elimination of the window also makes possible the counting of tritiated compounds in the solid state (7).

### *Determination of Tritium*

For the counting of tritium, gas phase assay is undoubtedly the most sensitive and accurate, notwithstanding the development of the internal gas-flow counter and scintillation methods referred to above and in Chapter VIII. Self-absorption reduces the efficiency of the internal gas-flow method from 50 per cent, which is expected from geometry alone, to 2.6 per cent. Eidinoff and Knoll (7) observe that their gas-flow counter method will normally be applicable only to highly active compounds and that labeled metabolites isolated from biochemical systems will usually have to be assayed by gas phase methods.

### *Assay of Compounds Containing Several Isotopes*

There are a number of elegant methods reported in the literature for the separate assay of two radioactive isotopes contained in the same compound. These usually take advantage of markedly different properties possessed by the radiations emitted by the two isotopes. For example, a thin-window counter filled with helium is 30 times as sensitive to the radiations from  $\text{Fe}^{59}$  as to those from  $\text{Fe}^{55}$ , whereas one with a thick beryllium window and filled with argon is sensitive only to the radiations from  $\text{Fe}^{55}$ . This is because the two radiations differ both in character and in energy. Those from  $\text{C}^{14}$  and tritium also differ markedly, but only in maximum energy; hence although  $\text{C}^{14}$  may be counted in the presence of tritium by means of a thin-window counter, the separate counting of tritium in the presence of  $\text{C}^{14}$  is not possible, as only the total radiation can be measured by means of a flow-type counter. Even then, owing to the difference in efficiency of



counting of the two isotopes in the flow counter and the difference in efficiency between the two kinds of counter, the calculation would be somewhat laborious and inaccurate. Combustion and separate assay of the carbon dioxide and water by gas phase methods is much more reliable. If the stable isotopes  $C^{13}$ ,  $N^{15}$ , or deuterium are also to be determined, combustion and gas phase manipulation cannot of course be avoided;  $C^{13}$  and  $N^{15}$  are determined in the mass spectrometer as carbon dioxide and elemental nitrogen, respectively, and deuterium is determined either in the form of water or as hydrogen gas.

### *Economy of Labeled Substrates*

In addition to the advantages already enumerated, gas phase assay makes possible a 10- to 100-fold reduction in the amount of labeled material used in an experiment. This may not alone always justify the use of a method that would not otherwise be used, but it is still a relevant consideration in many circumstances. Firstly, even a 10-fold reduction in the cost of labeled materials is not to be neglected when substrates cost as much as £200 (\$560) per millicurie (e.g. 1- $C^{14}$  glycerol). A large reduction in the amount of activity used also brings about a corresponding reduction in health hazards to the workers and may even permit of experiments on human subjects. Furthermore, in some experiments, for example, with labeled hormones, the specific activity of the available material may be such that the dose used contains an activity so low that the most sensitive methods of detection are essential if the experiment is to succeed.

### *Technical Difficulties*

Although the arguments in favor of the superior accuracy and sensitivity of gas counting methods over all others seem incontrovertible, it must also be noted that more skill is needed for the successful running of a gas analysis apparatus and, at least compared with the solid counting of uncombusted samples containing only  $C^{14}$ , the method is more lengthy. A considerable knowledge of high-vacuum technique is desirable, and some workers, particularly those trained only in the biological sciences, may

consider the learning of what almost amounts to a new discipline more trouble than it is worth, or, having mastered the technique themselves, may find it difficult to secure the services of assistant staff with the necessary capacity for using and maintaining relatively fragile apparatus. There is also a great investment of time and money in gas phase assay equipment—an investment which is justified only if the equipment is to be kept in continuous use. In the author's laboratory, this condition is satisfied and in fact it has been found necessary to arrive at a compromise whereby the burden of work on the gas analysis apparatus is somewhat diminished. This is achieved by carrying out small scale pilot experiments using end-window solid counting of uncombusted samples, followed, if preliminary results justify it, by more extensive experiments using doubly or triply labeled substrates ( $C^{13}$ ,  $C^{14}$ , and tritium) to which gas analysis technique is applied.

## REFERENCES

1. C. ENTENMAN, S. R. LERNER, I. L. CHAIKOFF, and W. G. DAUBEN, *Proc. Soc. Exptl. Biol. Med.* **70**, 364 (1949).
2. M. CALVIN, C. HEIDELBERGER, J. C. REID, B. M. TOLBERT, and P. E. YANKWICH, "Isotopic Carbon," Chapman and Hall, London, and John Wiley and Sons, New York, 1949.
3. R. F. GLASCOCK, *Biochem. J.* **52**, 699 (1952).
4. R. ABRAMS and L. CLARK, *J. Am. Chem. Soc.* **73**, 4609 (1951).
5. F. E. KELSEY, *Science* **109**, 566 (1949).
6. P. E. DAMON, *Rev. Sci. Instr.* **22**, 587 (1951).
7. M. L. EIDINOFF and J. E. KNOLL, *Science* **112**, 250 (1950).
8. J. SHARPE and D. TAYLOR, *Proc. Inst. Elect. Engrs.* (London) **98**, II, 209 (1951).

## CHAPTER II

### HIGH-VACUUM APPARATUS

#### GENERAL REQUIREMENTS

Gas phase assay of the radioactive isotopes of carbon and hydrogen necessitates the quantitative manipulation of gas samples, ranging from as little as 10  $\mu$ l. to as much as 100 ml., and also the evacuation to very low pressures of the counters and ionization chambers used for radioactive measurements. This is not only to permit the introduction of the measured sample to be assayed but because counters, in particular, are very sensitive to the presence of the air gases which interfere with their counting properties. A source of high vacuum and the application of high-vacuum techniques is therefore inevitable.

One of the chief advantages of gas phase assay over solid counting methods lies in its high efficiency and the consequent possibility of using activities smaller by a factor of at least ten; elaborate health precautions are therefore unnecessary. Nevertheless, the equipment should be housed in a large, well-ventilated laboratory provided with an extraction fan capable of changing the air several times an hour. Alternatively, if desired, the vacuum apparatus can be housed in a large cabinet with extraction fan and sliding glass doors. The temperature of the laboratory should be maintained at about 20°, which is not only comfortable for the workers but is a temperature at which vacuum grease is neither too stiff nor too fluid.

If it is possible to design the laboratory right from the start, it is an advantage, when a permanent installation is planned, to provide a bench lower than the usual laboratory bench by about 6 in. and accessible from three sides. This makes it possible for workers of average height to reach all stopcocks on the apparatus without uncomfortable stretching, with consequent risk of break-

ages, and permits the scaffolding to be anchored on the wall. Other benches in the laboratory should be at the usual height of about 3 ft.

Whether or not a permanent installation is erected will depend partly on the amount of use it is to be put to. Some workers, for example Anderson, Delabarre, and Bothner-By (1), recommend a system of combustion tube, traps, manometers, and vacuum line that can be assembled and disassembled by means of standard taper joints, thus facilitating cleaning or repair. Others, including the present author (2) find that, with reasonable care, cleaning is rarely necessary, and they prefer a permanent installation, finding it more robust, more easily handled, and, having fewer movable parts, more convenient than one which is demountable. Where it is to be used for routine assay of carbon and hydrogen isotopes, an all-purpose apparatus such as that set out in Fig. 1 is very useful and will be referred to in greater detail from time to time later in this book.

### *Pumps*

If the maximum use is to be derived from a gas analysis apparatus, it should be capable of being evacuated to a pressure of  $10^{-6}$  mm. or less. Anderson and co-workers (1) disagree with this and state that "high vacuums are not only unnecessary but undesirable. Use of a diffusion pump backed by a mechanical pump caused the analyses to be very erratic, possibly because the glass surfaces of the apparatus were desorbing gases in some unreproducible manner under these conditions." The present author has found that a permanent apparatus can be pumped out thoroughly with diffusion pumps and kept evacuated so that there is absolutely no interference by desorbed gases. The use of a high vacuum of the order of  $10^{-6}$  mm. mercury makes possible the rapid quantitative transfer of small volumes of gas and, by means of a McLeod gauge, their accurate measurement. In the present author's experience, diffusion pumps are an indispensable part of the equipment.

Large volumes of air are not normally passed through the pumps, and hence high displacement speeds are not essential. A



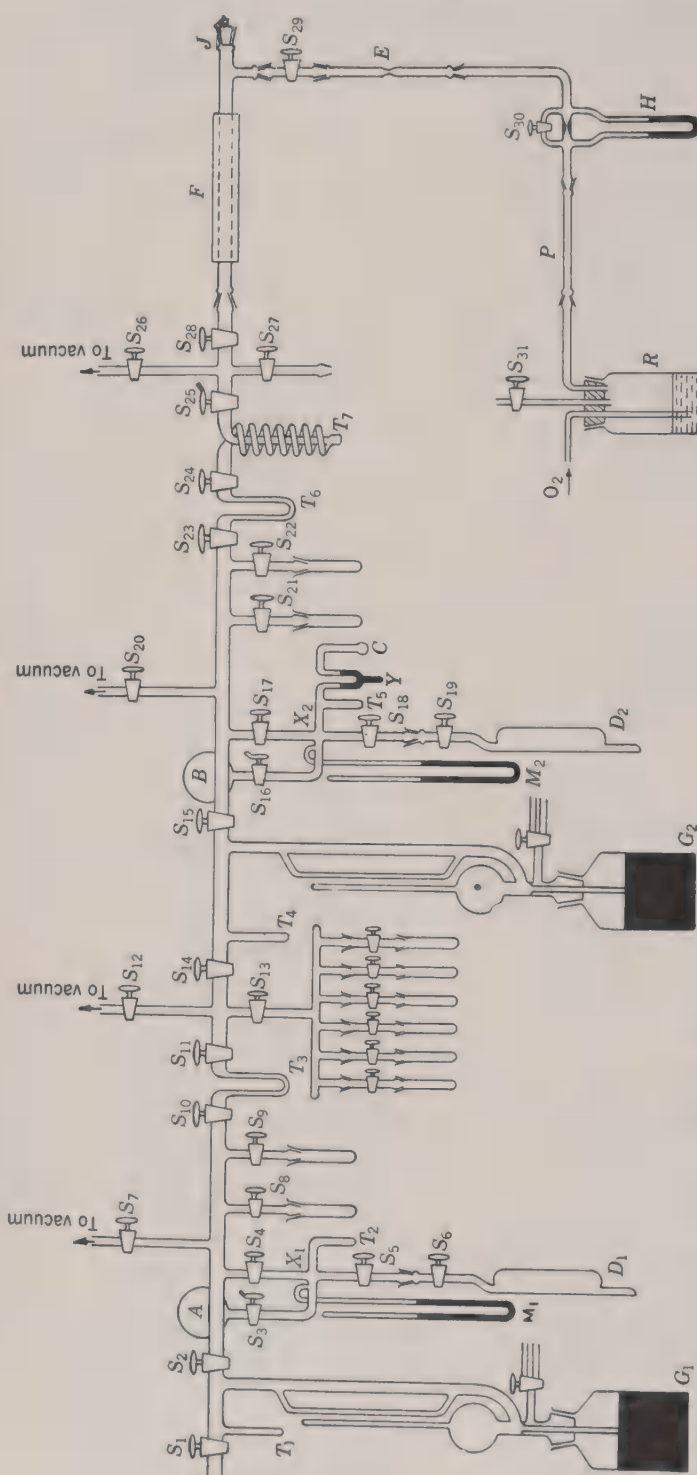


FIG. 1. Permanent high-vacuum line, showing lay-out of apparatus recommended by the author for the determination of tritium,  $C^{13}$ , and  $C^{14}$  in a single, 10-mg. sample.

A: Butane reservoir

B:  $CO_2$  reservoir

C:  $CS_2$  reservoir

$D_1$ : Stainless-steel-cathode gas counter

$D_2$ : Graphite-cathode gas counter

E: Oxygen purification tube

F: Furnace and combustion tube

$G_1$  and  $G_2$ : McLeod gauges

H: Flowmeter

J: Standard B14 taper joint and stopper

$M_1$  and  $M_2$ : Manometers

P: Platinized asbestos tube with B10 joints

R: Bubbler and oxygen reservoir

$S_1$ - $S_{31}$ : Stopcocks ( $S_{28}$  is "control stopcock," see text and Fig. 6b)

$T_1$ - $T_7$ : Traps and condensation tubes

$X_1$  and  $X_2$ : Manometric chambers

Y: Mercury cut-off (see Fig. 13)

Standard joints on side arms (beneath  $S_8$ ,  $S_{10}$ ,  $S_{31}$ , etc.) are all B10.



rotary pump giving an ultimate vacuum of 0.005 mm. mercury is used in the author's laboratory to back a mercury diffusion pump whose pumping speed is 10 l./sec.

The backing pumps should be housed in a sound-proof cupboard, or even outside the laboratory, because their noise can cause considerable nervous strain. They can be placed at some distance from the diffusion pumps providing they are connected by tubing of at least  $\frac{3}{4}$  in. diameter, made, preferably, of metal. It is particularly convenient if they are housed in racks, one above the other, to facilitate inspection and servicing.

Both mercury and oil diffusion pumps are commercially available, but the present trend is towards oil because oil diffusion pumps have a higher pumping speed for their size and because oil, being less volatile than mercury, is less liable to diffuse back into the apparatus. Thus, on many high-vacuum installations, the use of a liquid-air trap can be dispensed with if an oil diffusion pump is used. In the isotope laboratory, however, it is advisable to include the liquid-air trap with either kind of pump as a precaution against radioactive vapors accidentally introduced into the vacuum mains. It is to be noted that oil is much more readily contaminated than mercury, not only with organic vapors but with radioactive hydrogen itself which is not held back by a liquid-air trap. Hence it seems on balance that mercury vapor pumps are to be preferred on the isotopic gas analysis apparatus, notwithstanding the fact that it has been reported by Robinson (3) that mercury vapor interferes with the operation of a methane proportional counter; counters can always be protected from mercury vapor by means of a trap cooled to  $-78^{\circ}$ .

Diffusion pumps are available made of either metal or glass, and many workers choose metal because they are less easily broken. Steel pumps, of course, are easily damaged by water. Hence any water frozen on the inner surface of the liquid-air trap must be sublimed away and removed before shutting the apparatus down for the night, so that it cannot drip into the pump on melting. All types of diffusion pumps should be protected against a failure of the cooling water supply. Pump manufacturers make devices (e.g., Edwards' "Flowtrol") which automatically

switch off the current to the pump-heating elements if the rate of flow of cooling water falls too low.

### *Scaffolding*

A permanent high-vacuum apparatus should be erected on scaffolding of which several types are commercially available. Alternatively, the scaffolding may be specially constructed, pre-

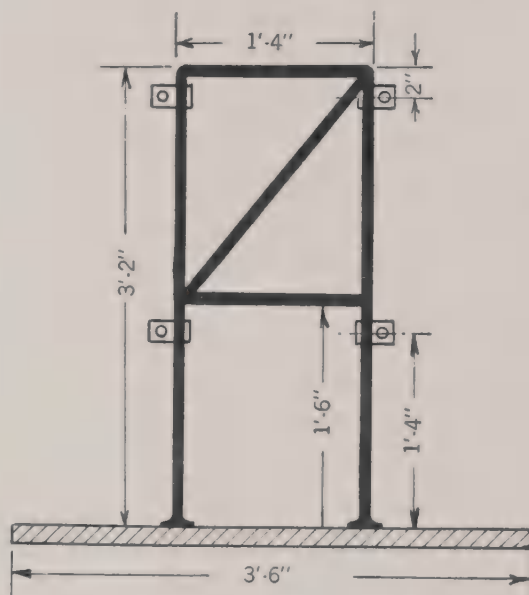


FIG. 2. Scaffolding unit. This unit may be made from  $\frac{1}{2}$ -in. mild steel or aluminum. Dimensions approximate.

ferably of  $\frac{1}{2}$ -in. aluminum rod, which is light, strong and corrosion-resistant. Figures 2 and 3 show the design of scaffolding units used in the author's laboratory. The chief points to be borne in mind when designing such a unit are that it should be tall enough for the working vacuum line to be at a height above bench level greater than that of the mercury barometer (see Fig. 4) and that it should be so rigid that no movement likely to cause breakage of the glass apparatus can occur. For the same reason, the bench top should be of massive, well-seasoned wood, not liable to warp or shift; alternatively, and better, the scaffolding can be supported on stout metal girders housed below the bench

and sunk in concrete foundations. The annoyance and delays caused by "spontaneous" fracture of the apparatus once assembled cannot be overemphasized.

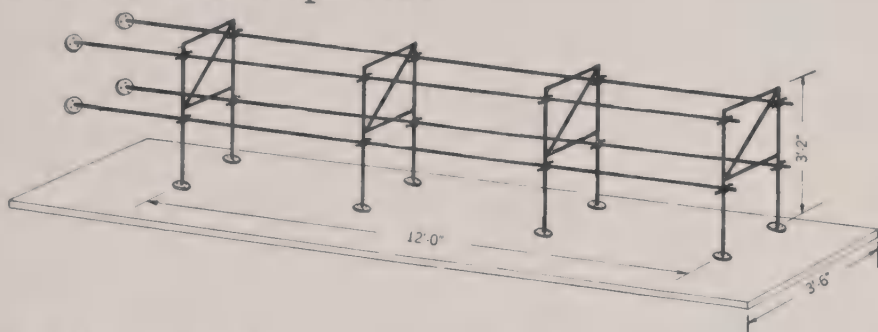


FIG. 3. Erected scaffolding for permanent high-vacuum line. Four units of the type shown in Fig. 2 are shown here screwed to the bench and connected by horizontal rods which are anchored on the wall.

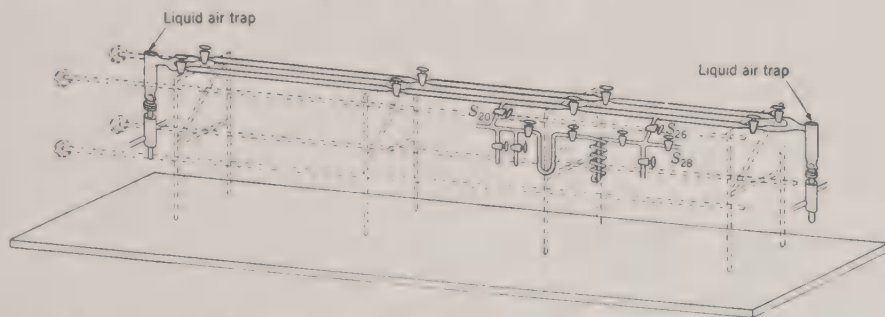


FIG. 4. Permanent high-vacuum line, showing vacuum mains and part of working line assembled on scaffolding.

### Stopcocks

High-quality vacuum stopcocks are essential, and it may be difficult to get really satisfactory ones that can be put into service without regrinding. There is quite a variety of patterns. Those most used in high-vacuum work are the oblique-bore and straight-through types (Fig. 5). Stopcocks of the oblique-bore pattern are less likely than the others to leak across the bore, and they have only one open position. The straight-through type, however, is always open when the handle is parallel with the side arms, which means that the setting of the apparatus can be seen at a glance. They are also somewhat easier to degrease than the oblique type, especially if grease has got into the side arms. If well ground they are reliably vacuum tight.

Whichever pattern is decided on, hollow keys should be chosen in preference to solid ones. They are much less likely to fracture when warmed and are much lighter. It is important also to restrict the number of sizes in use to a minimum so that an

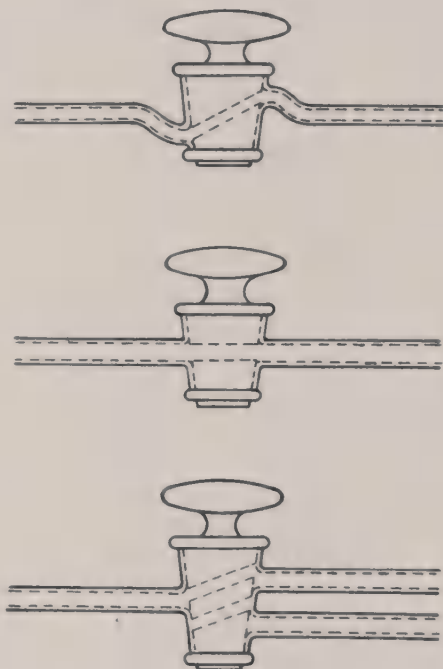


FIG. 5. Types of stopcock recommended for use on high-vacuum apparatus.

excessively large stock of spares need not be carried. The following sizes are in use in the author's laboratory:

- 15-mm. bore..... vacuum mains
- 10-mm. bore..... connecting working line to mains; e.g.,  $S_7$ ,  $S_{12}$ ,  $S_{20}$ ,  
and  $S_{26}$  in Fig. 1
- 8-mm. bore..... on working line; e.g.,  $S_{10}$ ,  $S_{11}$ ,  $S_{14}$ , etc.
- 6-mm. bore..... on side arms; e.g.,  $S_4$ ,  $S_8$ ,  $S_9$ ,  $S_{13}$ , etc.
- 3-mm. bore..... leading to gas reservoirs; i.e.,  $S_3$ ,  $S_{16}$ .

In addition, some solid-key, capillary, side-arm, 1- to 2-mm.-bore stopcocks are needed on mercury cut-offs, and some double-oblique (Fig. 5) of 3-mm. bore are needed for McLeod gauges and other air-pressure-operated instruments.



When a stopcock is received from the manufacturers it should have a piece of thin paper between the ground surfaces to protect them from the damage which will occur if they are turned together while dry. Both ground surfaces should be free from obvious scratches or blemishes, and the key, when inspected end-on, looking towards the light, should appear perfectly conical and not barrel-shaped. This stipulation is far from superfluous, and the author has rejected many stopcocks from inspection alone. It is well to be particular to the point of fussiness about stopcocks because a leaky stopcock can be a constant source of trouble.

*Greasing and testing.* Before building into the apparatus, stopcocks should be greased and inspected. The following method of greasing is to be recommended both for stopcocks under test and in service. The object should be to get a uniform film of grease between the ground surfaces without the inclusion of air bubbles and without getting surplus grease into the stopcock bore or side arms. Apiezon M or N is suitable, and, after gently warming the tube until the grease squeezes out in a semi-liquid state, six neat lines of grease are applied to the key in the manner indicated in Fig. 6a. The key is warmed gently in a stream of warm air until the grease becomes first transparent and then semi-liquid. The key is then inserted into the barrel in the open position and squeezed home. If sufficient grease has been used, it will flow evenly between the ground surfaces giving the whole stopcock a pale straw color. If too much grease is used, it will appear yellow and will gradually squeeze out under vacuum, much of it into the bore and side arms. The key is rotated several times to ensure uniform distribution of the grease, but it must not be turned until spreading is complete, otherwise striations will appear. If insufficient grease has been used and it will not cover the ground surfaces, it is useless to work the stopcock, as this procedure always results in striations in the grease film across which leakage can take place. It is also inadvisable to warm the barrel, especially on a stopcock in service on the apparatus, as it may result in grease running into the line.

A stopcock under test is greased in this way and set aside until



the grease has cooled to room temperature. If the key is then rotated gently, no striations should appear—especially in the region of the bore. A few at the extreme edge can be neglected, but striations near the bore invariably mean leakage. The stopcock must therefore be degreased and reground if it is to be used.

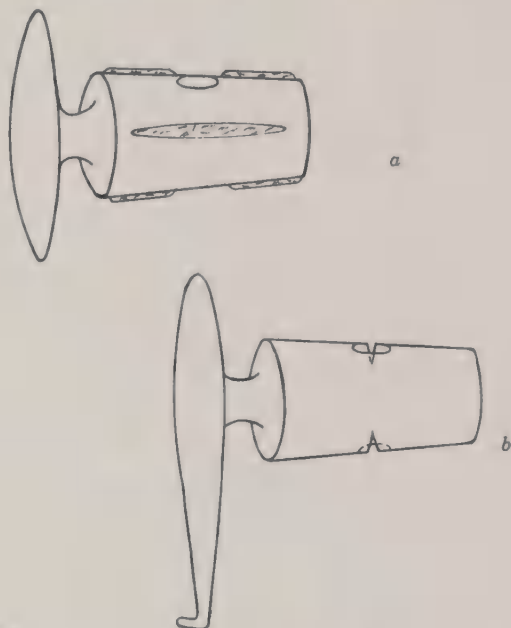


FIG. 6. (a) Procedure for greasing a stopcock. Six lines of grease are applied to the key from a collapsible tube. The key is warmed and inserted into the stopcock barrel in the open position. (b) Control stopcock. Stopcock modified by shallow file cuts on diagonally opposite sides of bore. Lengthened handle makes for easier control.

Regrinding is carried out with 700-mesh Carborundum made into a thin paste with water. The paste is applied to the key, and grinding carried out in the usual way, frequently withdrawing the key and reinserting to avoid uneven grinding. It is a tedious procedure and one on which it is difficult to give precise instructions. It is best to avoid it by securing a supply of stopcocks which do not need it. With practice it is possible to feel when

sufficient grinding has been done. The stopcock is then thoroughly washed under the running tap, grinding the wet surfaces together continuously to get rid of Carborundum. It is finally washed in distilled water, dried, and regreased for testing.

*“Control” stopcocks.* It is often necessary to admit gas very slowly from one part of the apparatus to another—for example from reservoirs *A* or *B* (Fig. 1) into counters, or from the combustion train into the trapping system (see Chapter VI). This is difficult to do with an ordinary stopcock without overshooting. If, however, the key of an ordinary vacuum stopcock has shallow file scratches made on diagonally opposite sides of the bore, as shown in Fig. 6b, then control becomes much easier. Stopcocks modified in this way are recommended for several parts of the apparatus, for example,  $S_3$ ,  $S_{16}$  and  $S_{25}$  (Fig. 1). It is convenient if the handle is lengthened, as shown in the figure, as this serves both to facilitate adjustment and to mark the fact that the stopcock has been modified.

### *Standard Taper Joints*

These need not be very large, and, for most purposes on a high-vacuum apparatus, B.10 joints are suitable, although bigger ones may be necessary for special purposes. As they are not normally subject to much movement, testing and regrinding is unnecessary, provided no “rocking” is possible when the two parts are united.

### *Traps*

A liquid-air trap of the kind illustrated in Fig. 7 is best for preventing back diffusion of mercury vapor and for protecting the pumps. It has the advantage over other kinds of traps in that a Dewar vessel to hold the liquid air is not necessary and it can be seen at a glance whether the trap needs replenishing. Such traps can either be bought ready-made from the makers of high-vacuum equipment or constructed in the glass-blowing shop from a desilvered Pyrex Dewar vessel of  $4 \times 20$  cm. internal dimensions to which standard taper joints are sealed. Vapor pumps can be bought provided with a metal flange adaptor of standard taper

which is bolted to the pump, the seal being effected with a rubber "O" ring. A convenient procedure which ensures a tight seal is to attach the flange adaptor to the trap before bolting it to the pump. The trap is held upside down in a clamp and the steel cone warmed in the hot air above a bunsen burner. A ring of high-

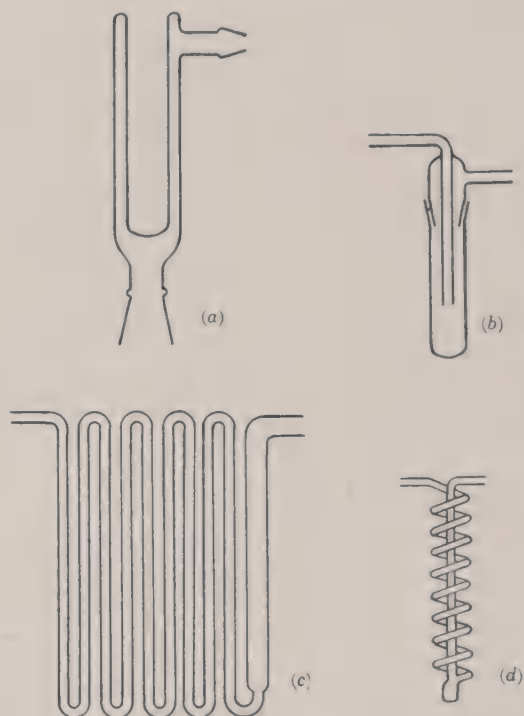


FIG. 7. High-vacuum traps. (a) Liquid air trap for protection of diffusion pumps. (b) Concentric trap. (c) Multiple U-trap of Buchanan and Nakao. (d) Spiral trap.

vacuum wax is applied to the cone which is then promptly inserted into the previously warmed socket on the trap. The wax should spread evenly between the metal and glass surfaces. More wax is run into the annular groove formed where the metal cone emerges from the glass socket. The flange should then be bolted firmly to the diffusion pump.

A concentric type of trap (see Fig. 7) should be inserted between the diffusion pump and the backing pump. This is not

normally chilled, but if water vapor is accidentally let into the line and collected on the liquid-air trap it can be removed by warming the trap up and allowing the water to pass through the diffusion pump into the concentric trap. The concentric type of trap can be also used elsewhere on the apparatus but, if fairly large quantities of gas have to be trapped, it is unwise to have the center delivery tube reaching right to the bottom as there is then a danger of it becoming blocked with solid gas. Effective trapping at low pressures can be achieved if the center tube reaches no more than half way to the bottom.

The concentric trap between the diffusion and backing pumps should be fairly large—at least 4 cm. wide and 20 cm. deep—and it will then serve also to prevent oil from being sucked back into the diffusion pump from the rotary pump if the latter is switched off and left without admitting air to it. This oversight happens in all laboratories from time to time.

Traps on the working line, for example those used for stripping the products of combustion from excess of oxygen, can either be simple U-traps, spiral traps, or concentric traps according to the conditions of use. Stripping a condensible component from a gas stream at atmospheric pressure is very difficult to do quantitatively: every gas molecule to be held must first come in contact with the cold surface and it may take a considerable length of tube to achieve this. Thus, Buchanan and Nakao (4), in their method for the wet combustion of organic compounds in an atmosphere of nitrogen, found that concentric traps chilled in liquid nitrogen failed to strip out the products of combustion at 60 cm. mercury pressure because of condensation of water and carbon dioxide in the gas phase to form smokes. They devised a multiple U-tube trap arranged so that revaporization of the smokes occurred during passage through the upper portion of successive loops, followed by condensation on the cooled glass of the next segment (see Fig. 7). Stripping of gases at low pressures, however, is very much easier and, at less than 0.1 mm. mercury pressure, quantitative stripping may be achieved by passing the gas mixture through a single, chilled U-trap. At somewhat higher pressures, of the order of 1 to 2 mm. mercury, a spiral trap may



be necessary. For example, in the author's laboratory, quantitative stripping of water and carbon dioxide from a stream of oxygen is achieved by a spiral trap and a simple U-trap in series, operating at a gas pressure of 2 mm. (2). The spiral trap is made from 1 meter of 3-mm.-bore glass tubing (see page 121).

### *Lay-out*

The whole assembly is best made of Pyrex glass because of its greater resistance to thermal shock and because it is easier than soft glass for the amateur glass blower to work. The most efficient way of providing vacuum when required to all parts of the ap-

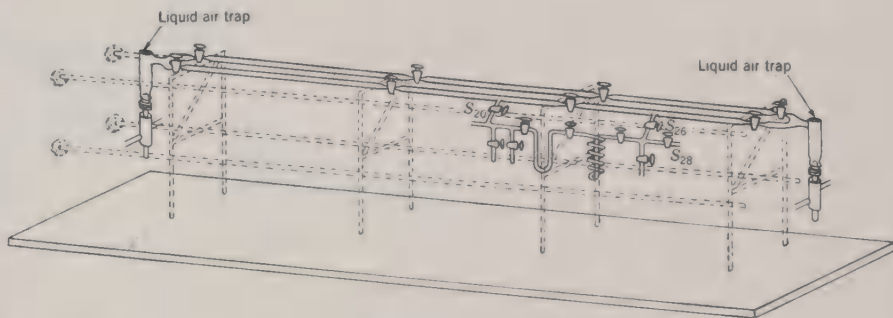


FIG. 4. Permanent high-vacuum line, showing vacuum mains and part of working line assembled on scaffolding.

paratus is by means of a ring vacuum main exhausted by at least two separate pumping systems. A large ring main makes it possible to lay the apparatus out in such a manner that a number of different operations can be carried out simultaneously in different parts of the apparatus without mutual interference. It is a great mistake to attach the vacuum pumps to one end of the working line and to string the traps, combustion train, and other pieces of apparatus along it; vacuum can then be applied to the further end only after clearing all the apparatus between it and the pumps. Furthermore, traps are usually of narrow-bore tubing, which so reduces the pumping speed that, in an installation thus arranged, irritating delays are inevitable.

A ring vacuum main supported on the metal scaffolding and

suitable for use on an island bench is illustrated in Fig. 4. The mains are fairly close together near the center of the bench and not vertically above the working line. This "pitched roof" arrangement makes for easy manipulation of the stopcocks connecting the working line to main, and yet leaves space immediately above the working line. The mains themselves are constructed of 25-mm.-bore tubing to permit high pumping speeds and rapid clearance of gas admitted to them. They are divided into six sections by stopcocks which, ideally, should have the same bore as the glass tubing of the mains; but 25-mm.-bore stopcocks are expensive and, unless frequently re-greased, require the application of considerable force to turn them. The author has therefore compromised on his apparatus by using 25-mm.-bore main tubing connected to 15-mm.-bore stopcocks. This inevitably reduces the pumping speed but not to an inconveniently low value.

The subdivision of the vacuum mains by stopcocks makes it possible to evacuate any section of the working line on either pump. The working line is laid out in 14–15-mm. tubing, held on the minimum number of clamps. Robust apparatus, such as McLeod gauges, gives some vertically directed support, and too many clamps are to be avoided as liable to cause strains and spontaneous fractures. The line must be at least 80 cm. above bench level; the mercury in McLeod gauges can then be allowed to run right to the top of the instruments without fear of flooding over into the line. Connections from the working line to the vacuum main should be fairly numerous; with the exception of U-traps, any part of the line that can be isolated by horizontal stopcocks should be provided with a separate connection to vacuum. Thus for example, in Fig. 1, section  $S_2$  to  $S_{10}$  is evacuated through  $S_7$ ,  $S_{11}$  to  $S_{14}$  is evacuated through  $S_{12}$ , and so on. A connection to vacuum on each side of a U-trap is also a great advantage since a condensable gas sample accidentally contaminated with air can then be recovered, wherever it may happen to be, by pumping slowly through the trap chilled in liquid air.

*Air lift and rough vacuum line.* McLeod gauges, mercury cut-offs, and other pieces of apparatus are operated by atmospheric pressure working against a vacuum, and are reset by a subsidiary

vacuum line which is best evacuated by a separate mechanical pump. In some laboratories the subsidiary line is evacuated by one of the main backing pumps, the diffusion pump being isolated by a stopcock while the pump is thus being used. This is feasible but inconvenient, and a subsidiary line with its own rotary pump is much to be preferred. The line should run down the center of the bench and about 4 in. above it. Apparatus on either side can thus be connected to it. It may also be convenient to run it to other parts of the laboratory for such uses as the evacuation of desiccators. The pump is protected by a concentric trap which should always be cooled to at least  $-78^{\circ}$  if there is danger of any water vapor getting into the line.

*Positional glassblowing.* It is common experience that the services of a professional glassblower can seldom be had at a moment's notice; hence if neither the scientist in charge of the isotope unit nor his assistants are reasonably competent glassblowers then considerable delays are likely to occur for only trifling alterations or repairs. The best arrangement is for the workers in the isotope laboratory to erect and maintain the high-vacuum apparatus themselves. For that reason, a short description of some of the salient points of the glassblowing technique then required may be appropriate here.

There are plenty of books and laboratory manuals with the help of which the novice can graduate from simple bends through T-pieces and internal seals to double-surfaces condensers, and it is not intended to repeat here such valuable instructions. Indeed, it is rare that such a difficult piece of glassblowing as the construction of a double-surface condenser is required in high-vacuum work. Rather, it is necessary to acquire a reasonable degree of skill with the hand blowpipe. This instrument is in more or less constant use in a gas analysis laboratory—not only for repairs and alterations but for sealing off samples of gas such as  $\text{N}^{15}_2$  and  $\text{C}^{13}\text{O}_2$  in break-seal tubes. Hence it is very useful to have a piped oxygen supply with frequent points laid round the laboratory. The hand blowpipe itself should have controls for both gas and oxygen within reach of the fingers of the hand that holds it



so that the other hand need never let go of the work to adjust them. These facts may be self-evident, but that does not prevent manufacturers from continuing to market blowpipes in which these requirements are not satisfied.

Much of the apparatus can be made in units at the bench blowpipe, but it must be assembled on the scaffolding. This often involves the insertion of a glass connection between two fixed points such as, for example, the insertion of a stopcock between the working line and the vacuum main. It may be accomplished as follows: First, the positions where the two seals are to be made are marked with a grease pencil, and holes are blown. It is best if these holes are somewhat smaller than the bore of the tube to be inserted and are reamed to size with the glass-working tool. This produces a useful shoulder to work to and ensures that the glass is reasonably thick and that tearing will therefore not occur when the seal is made.

The piece of glass to be inserted is cut slightly oversize. If the tube is of wide bore, then a clean cut may be achieved by making a circumferential scratch with a tungsten-steel knife, *moistening* the scratch and then applying a small intense blowpipe flame tangentially to it. A clean crack will usually follow the scratch. The connection is then sealed roughly into one of the two holes and, if properly cut to size, it should then come to within a millimeter or less of the other. The gap is closed by an operation known as "knitting." Pyrex glass needles are prepared by softening and thickening Pyrex rod and pulling out. The needle is then applied to the gap and glass worked in, blowing gently into the apparatus from time to time so that gaps can be found and plugged. This process becomes easy with practice. When the gap is knitted up, the whole joint is blown to shape, excess glass being pulled off with forceps. The other joint is then softened and blown to shape and, if necessary, the insertion aligned or straightened by softening both joints in rapid alternation so that movement can occur at both. Once this procedure has been mastered, the erection of extensive vacuum systems can be accomplished without difficulty.



## GAS MANIPULATION IN A VACUUM

High-vacuum technique is based on the fact that gases and vapors which exert a negligible vapor pressure at the temperature of liquid air or solid carbon dioxide can be rapidly and quantitatively moved about in a high vacuum by cooling the appropriate part of the apparatus. For successful operation of the technique, the tubes through which gas flow occurs must be reasonably wide (about 10 mm. diameter), and the apparatus must be pumped out so that air or other incondensable gases exert no appreciable pressure. This is particularly important if only small volumes of gas are to be dealt with; it would, for example, be impossible to transfer 0.5 ml. of carbon dioxide quantitatively from one section of the apparatus to another if air were present at a pressure as high as 0.5 mm. mercury because the mean free path of the carbon dioxide molecules would then be so small that a high proportion of them would not come in contact with the cooled surface within a reasonable time. Furthermore, it is obvious that, for accurate work, any contaminating incondensable gas must produce a reading in the instruments very much less than that produced by the sample being measured. Thus, as will be seen in the following pages, air at a pressure as low as 0.05 mm. would produce a reading in a McLeod gauge large enough to introduce a serious error into the measurement of 50–100- $\mu$ l. gas samples.

Gases which are incondensable at liquid-air temperature include oxygen and nitrogen, helium, carbon monoxide, and methane. All of these must therefore be manipulated by means of a Toepler pump used as described on page 43.

## GAS MEASUREMENTS

The simplest way of determining the volume of a gas sample is by measuring the pressure exerted in a fixed volume, the apparatus having been previously calibrated. The calibration may be carried out either by accurately determining the volume of the manometric chamber by weighing mercury or by using known volumes of gas and observing the pressure exerted.

### *Simple U-tube Manometer*

In the author's experience, manometers made from tubing of at least 3-mm. bore are preferable to those made from capillary tubing because less trouble arises from sticking and other surface effects. For most purposes it is unnecessary to construct a manometer reading from 0 to 760 mm. pressure, a range of 500 or 600 mm. usually being sufficient.

A piece of straight tubing is chosen, cleaned with chromic acid cleaning fluid, washed with distilled water, and dried. A constriction is then made approximately at the mid-point by thickening the tube in the blowpipe flame until the internal diameter is reduced to about 1 mm. This slows down the movement of the mercury and prevents it being driven with destructive force against the closed end of the manometer if the vacuum to which it is connected is accidentally broken. The tube is then bent into a U at the constriction, taking care that the limbs are coplanar. One end is sealed off and rounded in the blowpipe.

A convenient method of filling the manometer is as follows: An excess of clean, dry manometric-quality mercury is introduced into the bulb of the small flask shown in Fig. 8. It is attached to the high-vacuum apparatus by means of a standard taper joint. Both joint *J* and stopcock *S* are lubricated with silicone grease, and the stopcock on the high-vacuum apparatus protected from the subsequent heating by wet cotton wool. The flask must be supported in a clamp because of the weight of mercury.

The empty manometer is then sealed to the flask in the position shown in the figure and evacuated to a pressure of  $10^{-6}$  mm. or less. The mercury is heated gently until it is seen condensing on the neck of the flask and bubbles are forming in it. This operation, which dries and outgasses the mercury, must be carried out very carefully to avoid breaking the apparatus and scattering hot mercury in the laboratory. The manometer is gently flamed in the hand torch and pumping continued for about 1 hour to ensure that it is dry and free from adsorbed gas. A "black vacuum" should be observed when the high-frequency coil is applied.

When thoroughly evacuated, the stopcocks are closed, the whole device removed at the joint, and mercury tipped into the U-tube until it is about 2 cm. above the mid-point. Air is then carefully admitted to the flask, driving the mercury into the

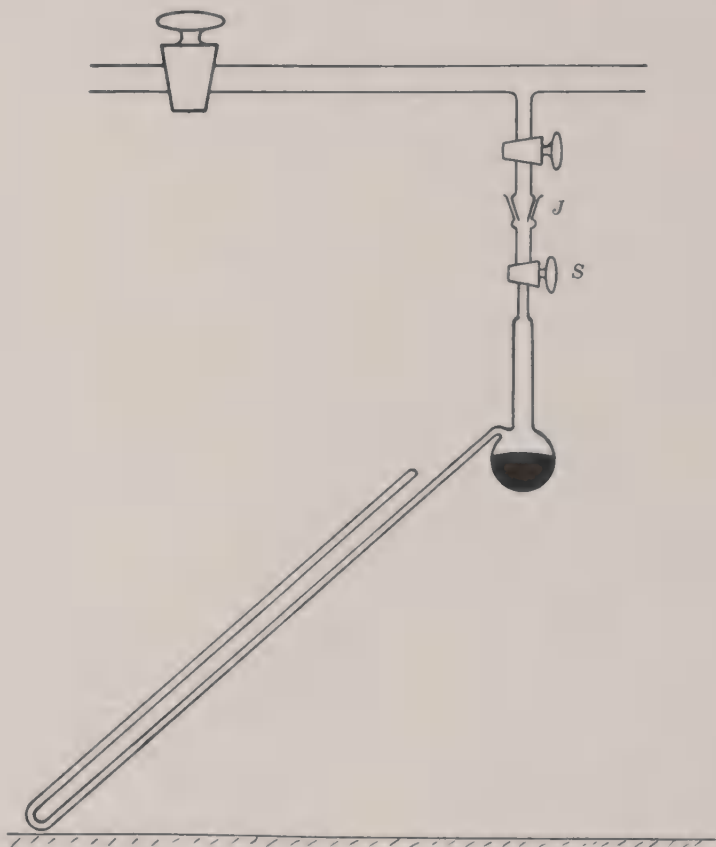


FIG. 8. Apparatus for filling manometer. The manometer is sealed to a bulb containing clean mercury and attached to the high-vacuum apparatus. It is thoroughly pumped out, warming both manometer and mercury to eliminate adsorbed and occluded gas. Stopcocks are then closed, the device removed from the apparatus at the joint, and the required amount of mercury tipped into the manometer.

closed limb and completely filling it. Not even the smallest bubble should be visible at the top of the closed tube, and the mercury in the open limb should be from 3 to 4 cm. from the bottom. The instrument, which is now rather fragile because

of the weight of mercury, should be attached to a boxwood or ivory support with suitable clips. A useful support is a meter rule, preferably engraved with a zero in the middle. Paper scales stuck to wooden supports are quite useless. Engraved, glass, mirror scales are the most accurate, and a pair of them arranged as shown in Fig. 9 are useful.

*Measurement of samples.* By means of a simple manometer connected to a manometric chamber of about 30 ml. internal volume, the measurement of gas samples in the range of 1 to 20 ml. may be achieved with a satisfactory degree of accuracy. The error is greatest at the lower end of the range where 1 ml. gas exerts a pressure of about 2.5 cm. If the mercury surfaces are clean, however, and moving smoothly over clean glass, it should be possible to take the reading to 0.25 mm., resulting in an error of not more than 1 per cent.

In Fig. 29 (page 84) is illustrated a manometric chamber of this type. It is bounded by stopcocks  $S_{16}$ ,  $S_{17}$ ,  $S_{18}$ , the manometer, and the mercury cut-off. The volume of the chamber cannot be measured directly, but a volume-pressure curve can be constructed by observing the pressure exerted by various accurately known volumes of gas. In the author's laboratory, there is a standard 50-ml. bulb whose internal volume at room temperature has been accurately determined by weighing mercury. It is fitted with a stopcock and standard taper joint. To calibrate a manometric chamber such as that illustrated, the standard bulb is attached below stopcock  $S_{18}$  and evacuated. Carbon dioxide (in this case from the reservoir) is then admitted to the bulb to a carefully read pressure  $p$ , the stopcock on the bulb closed, and the excess carbon dioxide in the rest of the apparatus pumped away. Then, if the internal volume of the bulb is  $V$  ml., and the laboratory temperature is  $T^\circ$  K., the bulb contains

$$\frac{273Vp}{760T} \text{ standard milliliters}$$

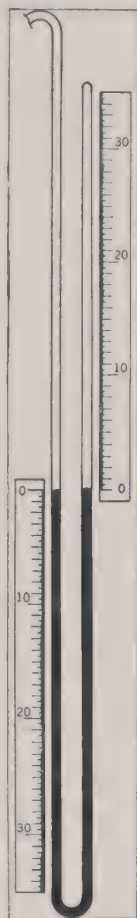


FIG. 9.  
Manometer.



Stopcock  $S_{17}$  is closed and the gas in the bulb transferred to the manometric chamber by chilling the trap  $T_5$  in liquid air and opening the intervening stopcocks. After closing stopcock  $S_{18}$  and allowing the gas to warm to laboratory temperature, the pressure which it exerts in the manometric chamber is recorded. A number of such observations are made over the range of pressures readable on the manometer and a graph of volume against pressure constructed. It is never quite linear, owing to the progressive slight increase in chamber volume with pressure due to displacement of mercury in the manometer.

### *Van Slyke Type Manometer*

The manometer used on the well-known Van Slyke manometric apparatus has only one graduated limb, the mercury in the other being always adjusted to the same fixed point by means of a leveling bulb. Buchanan and Nakao (4) use such a manometer for the measurement of radioactive carbon dioxide samples, and this part of their apparatus is illustrated in Fig. 10. The calibrated bulb  $n$  may be replaced by others, larger or smaller, according to the size of gas samples to be measured. Stopcocks  $S$ ,  $J$ , and  $K$  communicate with the rest of the apparatus and with high vacuum. The manometer consists of the limbs  $r$  and  $x$  as far as stopcocks  $T$  and  $U$ , and the leveling bulb  $V$ . The manometric chamber comprises the calibrated bulb  $n$  together with the limb of the manometer  $x$  as far as its zero  $O$ .

In order to measure a gas sample, the whole apparatus is first evacuated to about  $0.02 \mu$  with stopcocks  $S$  and  $J$  open to the pumps. The mercury in the manometer is adjusted by means of the leveling bulb, stopcock  $U$  being open, so that it reads zero simultaneously in both  $r$  and  $x$ . If it does not, the reason may be leakage of air into one limb, or bubbles in the mercury. The gas sample to be measured is then transferred to  $n$  using liquid air. Stopcocks  $K$  and  $J$  are closed and  $n$  is allowed to warm up with the leveling bulb  $V$  in a raised position and stopcock  $U$  open so that the mercury stands high in the limbs  $x$  and  $r$ . A Dewar vessel of water at laboratory temperature is then placed around  $n$  and the system allowed to come to equilibrium. Then,



by moving the leveling bulb *V* up or down, the mercury meniscus in *x* is adjusted to zero and the stopcock *U* closed. The pressure which the gas sample exerts in the manometric chamber is then read off on the graduated limb *r*.

As will be described later (page 88), this instrument with its movable leveling bulb is widely used on a portable apparatus for

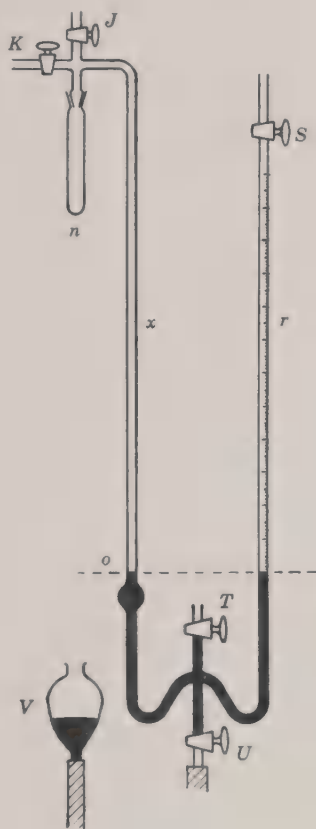


FIG. 10. Van Slyke type manometer (part of apparatus used by Buchanan and Nakao).

*J, K, S, U, T*: Stopcocks  
*n*: Interchangeable calibrated bulb  
*r*: Graduated manometer limb  
*o*: Zero level in limb *x*  
*V*: Leveling bulb

gas measurement without the use of pumps. On such pieces of equipment the leveling bulb is virtually indispensable. On a fixed apparatus provided with pumps, however, the leveling bulb is somewhat clumsy, and the mercury could be more conveniently raised and lowered by atmospheric pressure and a rough vacuum pump, respectively, as is described later in this chapter for the McLeod gauge. Van Slyke himself (5) has recommended connecting a pump to the top of the leveling bulb, thus avoiding

the need for raising and lowering it, but he still retains the loop of mercury-filled rubber tubing.

### *Two-liquid Manometers*

Although the measurement of gas samples smaller in volume than 1 ml. might be achieved by reducing the size of the manometric chamber, there are obvious limits to this procedure. It is better to devise a means of measuring low pressures accurately, and one method of doing so is by means of a two-liquid manometer such as that described by Anderson, Delabarre, and Bothner-By (1). This is essentially a device in which small, pressure-induced movements of mercury in a U-tube manometer are magnified many times.

The right-hand drawing of Fig. 11 shows the manometer filled for use. The manometric chamber is connected to *M* which communicates with a U-tube filled with mercury. An oil of low vapor pressure completely fills the space above the mercury in the right-hand bulb and about 2-cm. of the capillary tube above it when the manometer is at its zero setting—i.e., when *M* is fully evacuated. The instrument works on a principle similar to that of a thermometer in which a small volume change of the mercury in the bulb is magnified into a large linear movement of the mercury in the capillary. In this manometer, a small movement of mercury into the oil-filled bulb compresses sufficient oil into the capillary to produce a large movement of the oil meniscus. By adjusting the size of the manometric chamber, a wide range of gas volumes may be measured. Water itself may also be measured manometrically by allowing it to evaporate into a chamber of such a size that it exerts a pressure considerably less than its saturated vapor pressure (where deviations from the gas laws would cause considerable errors).

When using the instrument, care must be taken to ensure that the oil drains back to the zero mark between readings. By using a manometric chamber of about 12.5 ml. capacity to which, by opening a stopcock, another could be added of twice that volume, Anderson and co-workers found that they could measure samples of carbon dioxide containing from 0.3 to 6 mg. carbon.

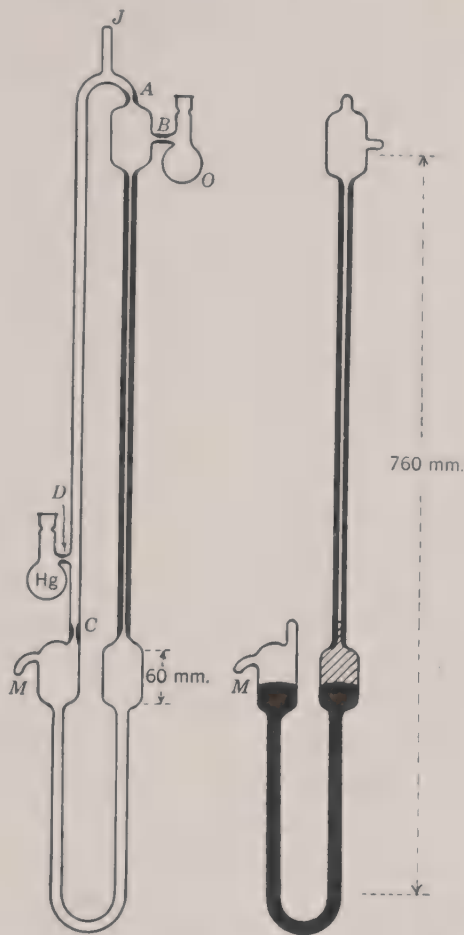


FIG. 11. Two-liquid manometer (Anderson, Delabarre, and Bothner-By). Left-hand drawing shows apparatus ready for filling, and right-hand drawing shows filled manometer after removal of accessory parts. *A*, *B*, *C*, and *D* are constrictions for sealing off; *O* is an oil-containing flask; *Hg* is a mercury-containing flask; *M* is the point of attachment of manometer to main apparatus (see Fig. 38).

The additional apparatus needed for filling the manometer is shown connected to it in the left-hand drawing of Fig. 11. Constrictions for sealing off under vacuum are provided at the points marked *A*, *B*, *C*, and *D*. The whole instrument is thoroughly cleaned, washed, and dried, and then connected to a high-

vacuum manifold at the spherical joint *J*. Clean mercury is placed in flask *Hg*, and dibutoxytetraethylene glycol in flask *O*. Both flasks and the inlet tube *M* are sealed or plugged. The manometer is evacuated and baked out by the careful application of a soft flame, and gas is expelled from the oil by repeated freezing and thawing.

When the pressure is reduced to  $10^{-4}$  mm., or less, mercury is distilled from flask *Hg* into the U-tube until it fills it completely together with the lower 2 cm. of the cylindrical bulbs. Constrictions *C* and *D* are then sealed off, the flask *Hg* being removed. Oil is distilled from flask *O* into the lower right-hand cylindrical bulb filling the space above the mercury and about 2 cm. of the capillary. Anderson and co-workers recommend this distillation procedure, but it seems likely that the same result could be achieved more quickly by tipping if a tap were provided at *J* so that the whole instrument could be removed from the manifold.

When the apparatus is cool, the oil should extend not more than 3 cm. into the capillary and the vacuum should still be of the order of  $10^{-4}$  mm. Constrictions *A* and *B* are then sealed off, the bulb *O* and supporting tube being removed leaving the manometer in the condition shown in the right-hand drawing of Fig. 11. It can be attached to the manometric chamber at *M* either by means of a standard taper joint or by sealing it permanently in position. The capillary tube is provided with a milk-glass centimeter scale and sliding vernier, making it possible to take readings with an error not exceeding 0.1%. A somewhat similar two-liquid manometer has been described by Naughton and Frodyma (6) as part of their apparatus for the micro-determination of carbon and hydrogen.

### *The McLeod Gauge*

The McLeod gauge is usually employed for the measurement of low pressures, but it is fundamentally a volume-measuring instrument and, as such, can be usefully applied to gas analysis. A convenient pattern is illustrated in Fig. 12. Since the instrument is kept evacuated except when small volumes of gas are admitted to it for measurement, the pressure in it never exceeds a

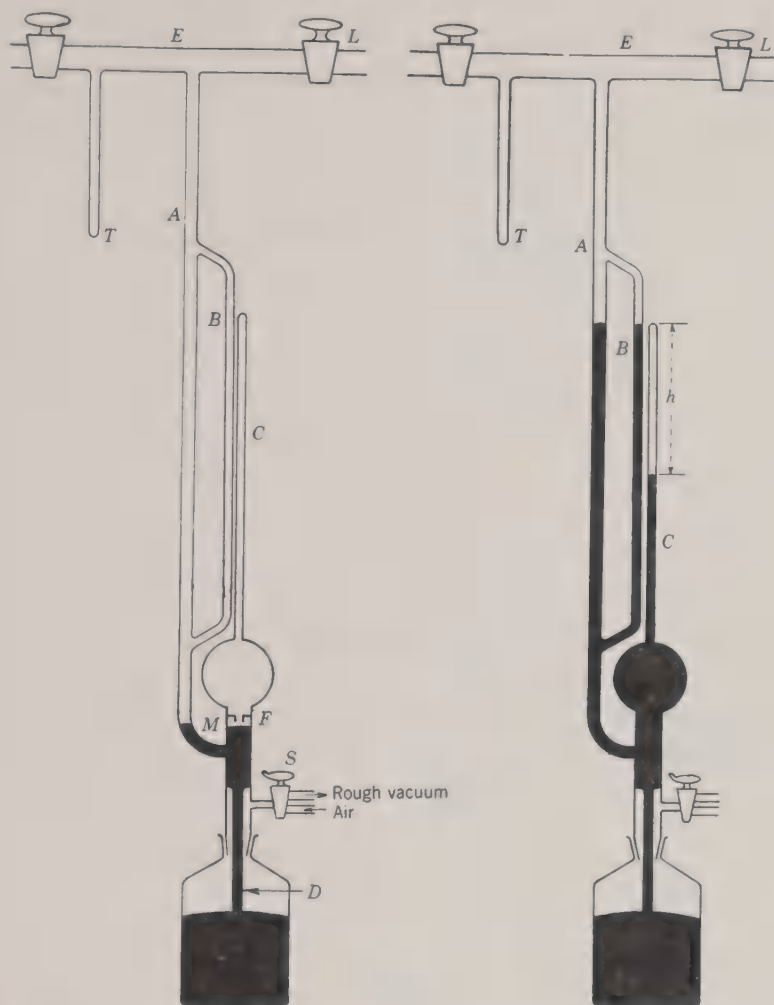


FIG. 12. McLeod gauge. Design recommended by author for gas measurements in the range 10–1000  $\mu$ l. Two views: one with mercury rising and one with mercury set for taking a reading. A: main limb; B: subsidiary limb to facilitate setting; C: calibrated tube; E-L: working line; T: trap for condensing sample into gauge; F: surface renewer. Other parts are referred to in text.

few millimeters of mercury. The mercury is raised or lowered by applying atmospheric pressure or rough vacuum to the mercury in the bottle by operating the two-way stopcock S. This method is considerably more convenient than the raising and lowering of a leveling bulb connected to D by means of rubber tubing.



If the McLeod gauge and bottle are evacuated and air then cautiously admitted to the bottle, mercury will rise in both limbs, displacing and compressing rarified gas from the bulb into the calibrated tube *C*. At pressures of  $10^{-6}$  mm. or less, the mercury will virtually fill the tube *C* when the levels in *A* and *B* are opposite the top of it because the bubble of gas in *C* is too small to see. If the mercury is allowed to continue rising, it will ultimately come to rest when it stands in *A* at the barometric height above the mercury in the bottle. Thus it is essential to have the working line *E-L* at a height above the bench some 10 cm. or so greater than that of the barometer so that flooding with mercury cannot occur through accidentally allowing it to rise to its full height.

For a brief description of how the McLeod gauge works, suppose a few hundred microliters of gas have been introduced into it and the stopcocks in the line *E-L* closed. The mercury is raised by admitting air cautiously to the bottle through the two-way stopcock, controlling it by means of a forefinger placed over the inlet. Compared with the rest of the instrument, the volume of the bulb is large and therefore most of the sample of gas will be trapped in it as the mercury passes the junction *M*. The mercury is brought to rest with the levels in tubes *A* and *B* exactly opposite the top of the tube *C*, as shown in the second drawing of Fig. 12. The purpose of the tube *B* is to enable this adjustment to be made accurately. The mercury level in *C* will be several centimeters lower because of the pressure of the trapped gas.

If the cross-sectional area of *C* is  $a$  cm.<sup>2</sup>, and the length of the gas column in *C* is  $h$  cm., then the volume of this column of gas at the pressure of the reading is  $ha$  cm.<sup>3</sup>.

The pressure of the gas is  $h$  cm. of mercury (since the pressure of the residual gas in *A* is negligible), hence the volume of gas at standard pressure is

$$\frac{ha \times h}{76} = \frac{h^2 a}{76} \text{ cm.}^3 \quad (1)$$

Thus the volume of gas in the calibrated tube, corrected to standard pressure varies, as the *square* of the reading ( $h$ ), and

this accounts for the wide range of volumes which may be accurately measured. For example, in a tube of 3 mm. inside diameter, a reading of 1 cm. corresponds to a volume of about 1 standard microliter, whereas a reading of 20 cm. corresponds to about 400 standard microliters. By making the tube *C* from tubing of a suitable size, a different range may be covered. Because the cross sectional area *a* varies as the square of the internal diameter, so, for a given length of tube, the volume of gas that can be measured will also vary as the square of the diameter. It is thus better to have McLeod gauges specially constructed for gas analysis work rather than to buy commercial models because these are usually designed for pressure measurement and are provided with calibrated tubes (*C*) whose capacity is much too small.

In the practice of McLeod gauge construction it is not usual to measure the internal diameter and calculate *a*. It is best to measure the internal volume *v* ( $= ha$ ) for various values of *h* by weighing mercury. Then if a reading is taken in the usual way, the volume of gas (*V*) in the tube, corrected to standard pressure, is given by  $V = vh/76$  or, at N.T.P. by

$$V = \frac{273vh}{76T} \quad (2)$$

where *T* is the absolute temperature at which the reading is taken.

If, therefore, before *C* is sealed into the instrument, a number of measurements of the internal volume *v* are made for corresponding values of *h*, and the value of *V* calculated in each case from equation 2, then a plot of *V* against *h* will give a parabolic curve on ordinary graph paper or a straight line on logarithmic graph paper.

For a reasonable degree of mechanical strength, the limb *A* should be made from tubing of internal diameter 8–10 mm. The gauge is then fairly robust and will give some vertical support to the working line and attached apparatus. The size of the bulb determines the sensitivity of the instrument because it determines, at any given pressure, the volume of gas trapped and com-

pressed into the calibrated tube. This however is most important when the instrument is being used to measure low pressures; for the measurement of volume it is necessary only to ensure that the volume of the bulb accounts for 75 to 85 per cent of the internal volume of the whole instrument. Thus, when *A* is made from 10-mm. tubing and the line *E* from 15-mm. tubing, a bulb of from 100 to 150 ml. capacity is suitable. The tube *B* should be made of tubing of the same internal diameter as *C*, the size of which is determined by the range of measurable volumes to be covered.

The mercury reservoir bottle may be either an ordinary laboratory bottle of suitable capacity, chosen so that the ground neck is sufficiently similar to a standard taper joint for them to be ground together to fit, or it may be a heavy-walled flat-bottom or conical flask with standard taper joint neck. Whichever is chosen, it must be strong enough to support both atmospheric pressure when it is evacuated and the weight of about 150 ml. (4 lb.) of mercury on its neck.

The tube *D* should reach to within a few millimeters of the bottom of the bottle and its end should be reduced in internal diameter to about 1 mm. by heating in the blow-pipe flame. This reduces the rate of flow of mercury into the bulb which otherwise may occur with almost explosive violence if air is admitted too suddenly to the bottle when the McLeod gauge is in use. A surface breaker (*F* in Fig. 12) may also be included if desired to ensure that only a fraction of the surface of the rising mercury gets into the bulb.

*Calibration of tube.* The tube *C*, which should have its closed end flattened internally by heating in the blowpipe flame, is calibrated by weighing mercury. Table 2 shows the figures obtained from an actual calibration in the author's laboratory. The tube was filled with clean, dry mercury to within a few millimeters of the top, and tapped to expel air bubbles. The length of the column was then carefully measured, some of the mercury tipped into a previously weighed bottle, the remaining column of mercury measured, and the bottle reweighed. This procedure was repeated at intervals until no more mercury remained in the tube. The figures obtained are shown in the first two columns of

Table 2. The last pair of readings in the table correspond with the tube empty and all the mercury in the bottle.

TABLE 2

Laboratory temperature = 21° C. = 294° K.				
<i>Length of Hg column = h (cm.)</i>	<i>Weight of bottle + Hg (gm.)</i>	<i>Actual weight of column (gm.)</i>	<i>Volume of column = v (ml.)</i>	<i>Volume of v ml. gas at pressure h cm. corr. N.T.P.</i>
17.00	22.541	19.677	1.453	0.3018
13.95	26.106	16.112	1.190	0.2028
11.59	28.850	13.368	0.988	0.1400
8.20	32.783	9.435	0.697	0.0701
4.60	36.981	5.237	0.387	0.0218
2.18	39.756	2.462	0.182	0.0049
1.14	40.917	1.301	0.096	0.0013
0.00	42.218	0.000	0.000	0.0000

The actual weight of mercury in the tube each time a measurement was taken was found by subtracting the corresponding weight of bottle and mercury from the last weighing. Thus, when the length of the column was 17.00 cm., the bottle was empty and weighed 22.541 gm. The weight of the bottle together with all this mercury was 42.218 gm. (last entry) and the difference (19.677 gm.) was therefore the weight of the column. Similarly, the weight of a column 13.95 cm. long was found by deducting the corresponding weighing (26.106 gm.) from 42.218, and was 16.112. These weights are shown in the third column. The volumes occupied by these weights of mercury were obtained by dividing by the density of mercury at the temperature at which the readings were taken (13.54 gm./ml.) and are given in the fourth column. These values therefore gave the internal volume  $v$  of the tube for various values of  $h$  and could be substituted in the equation

$$V = \frac{273vh}{76T} \quad (2)$$



where  $V$  was the volume in standard milliliters of gas when the McLeod gauge was used in the prescribed manner. These values of  $V$  (converted to microliters) were plotted against the corresponding values of  $h$  on log-log, graph paper and a straight line obtained from which could be read the volume in standard microliters for any reading of the instrument.

In general it is advisable to calibrate the tube and plot the graph before proceeding any further with assembly and erection. Should any point not lie on the same straight line as the others, further values in its neighborhood should be obtained because a deviation from linearity may be due to the internal diameter of the tube not being uniform. If this is found to be so then the tube must be rejected.

*Erection and operation.* The calibrated tube is sealed to the bulb and the instrument aligned, making sure that the tube is coaxial with the bulb, that tubes  $A$ ,  $B$ , and  $C$  are coplanar, and that all stand vertically when the gauge is plugged into the reservoir bottle and stood on a flat surface.

It is most important to ensure that all inner surfaces are thoroughly clean. The McLeod gauge is therefore filled with freshly prepared chromic acid cleaning fluid and left for several days. It is then washed thoroughly with tap water and, finally, many times with distilled water taken, preferably, straight from the still and not from a laboratory storage container or wash bottle. In the author's laboratory, the regular procedure is to fill and empty the gauge at least six times with distilled water before finally shaking as much out as possible and allowing to drain. It is assembled into the high-vacuum apparatus wet; under no circumstances should it be dried by washing with acetone or alcohol and ether. Solvents invariably leave a greasy film behind which will entirely nullify the effect of the preceding cleaning and greatly interfere with the smooth movement of mercury surfaces.

The mercury also must be scrupulously clean and the best triple-redistilled manometric quality should be used. More than sufficient to fill the McLeod gauge is filtered through a perforated paper into the bottle, and it is therefore useful to have previously

ascertained the volume of the instrument by weighing full of water. The gauge is inserted into the bottle, and the joint made tight with high-vacuum wax.

Three buttons of melted wax are then poured on the bench in the previously marked position, and the bottle, also gently warmed, is placed in position and bedded down. This ensures rigid support without the danger of rocking. The end of the tube *A* is sealed into the line between a pair of stopcocks about 20 cm. apart and the trap *T* added. One of the arms of the two-way stopcock *S* is sealed into the rough vacuum line and the key of the stopcock marked for future identification by drawing a pip on one end of the handle in the blowpipe flame. If all such stopcocks in the laboratory are marked in the same way—i.e., so that the pip is always in the same position when the bottle is connected to vacuum—manipulation is greatly facilitated since the setting of the stopcock can be felt and immediately adjusted without looking at it.

The pumps are now protected by means of a trap cooled with solid carbon dioxide and the McLeod gauge cautiously evacuated. This is accomplished stepwise. Some air is withdrawn and the mercury allowed to rise as far as *M*. Air is then withdrawn from the bottle so that the mercury falls to its original position. This is repeated until there is a vacuum in both bottle and McLeod gauge. If either side is evacuated too far without maintaining this balance, some inconvenience may result; if the mercury rises past *M* before evacuation is complete, air will bubble from the bulb into *A*, perhaps splashing mercury as far as *E* and into *T* and the stopcocks; if the bottle is evacuated too quickly, air will bubble from the gauge through *D*, causing violent bumping which may break the instrument or splash mercury into the rough vacuum line.

When both sides are evacuated, the gauge is left on the vacuum pumps until all the water has been evaporated. It is then gently heated with a soft flame and, when cool, the mercury run up and down several times. It will take about a day's pumping before a hard vacuum is obtained, as evidenced by the rising mercury striking the top of *C* with a distinct click. Prolonged

pumping and flaming is necessary before a "sticking vacuum" is obtained i.e., when the mercury in *A* and *B* can fall several centimeters below the top of *C* before the mercury filling *C* falls also.

*Taking measurements.* If a sample of gas is introduced into the instrument and the mercury run up in the usual way, the length of the gas column and reference to the calibration graph will give the volume of gas (in standard microliters) contained in the calibrated tube. It will not, however, take into account the gas in the dead space ( $A + E + T$ ). If all that is required is a sample of known size, then the gas in the dead space may be pumped away while the mercury is up, and the measured volume in *C* taken for assay. It is often useful, however, to know the volume of gas in the whole instrument, and this is obviously greater than that measured in *C* by a constant factor which is independent, or nearly so, of the volume of gas taken. This factor may be determined by a very simple procedure. A sample of gas is introduced into the gauge, the mercury run up, and the dead space pumped out. The volume in the calibrated tube is measured and the mercury lowered, allowing this gas to expand into the whole instrument. The mercury is then run up again and the volume in the calibrated tube measured without pumping away the gas in the dead space. The reading will now be less. The ratio of the two volumes is the factor by which the volumes measured in the calibrated tube must be multiplied to give the volume of gas in the whole instrument. The mean of several such determinations should be taken and marked on the instrument in indelible ink. The factor is usually of the order of 1.25.

*Elimination of water vapor in gas measurement.* Although perfectly dry gases manipulated in a dry apparatus can be accurately measured by the above procedure, in ordinary routine use it will usually be found that small quantities of water vapor are picked up from parts of the apparatus previously exposed to moist gases. The water vapor is apparently desorbed from the glass and condensed, together with the sample of gas, in the trap where it is measured. It usually amounts to no more than 10–20  $\mu$ l. and does not introduce an appreciable error into ordinary manometric measurements; it is large enough, however, to intro-



duce serious errors into measurements with the McLeod gauge. The passage of gas samples through U-traps cooled to  $-78^{\circ}$  will not remove it, but accurate measurements can be taken if the liquid air used to condense the sample into the McLeod gauge trap is replaced with a bath at solid-carbon-dioxide temperature. Under these conditions the water remains condensed and an insufficient amount enters the gas phase to cause a measurable error. This procedure is recommended for the routine measurement of carbon dioxide, butane, and any other gas which will not be condensed at  $-78^{\circ}$  at the pressure obtaining within the gauge.

### ADDITIONAL APPARATUS

#### *Toepler Pumps*

For the manipulation of incondensable gases such as hydrogen, methane, and nitrogen, a Toepler pump is commonly used. One very useful form can be made by the addition of a stopcock and standard taper joint to the top of tube *C* of the McLeod gauge shown in Fig. 12. Gas in the bulb can then be expelled through the stopcock into a previously evacuated sample tube by raising the mercury. The stopcock is closed and the mercury lowered past the junction *M*, when more gas from the line will expand into the bulb. The mercury is then raised again and another portion of the gas expelled through the stopcock attached to the top of *C*. The number of strokes necessary to achieve virtually quantitative transfer of gas depends on the relative volumes of Toepler bulb and the apparatus from which gas is being pumped. Thus, if the gas is contained in a piece of apparatus of the same volume as the Toepler pump, five strokes will transfer nearly 97 per cent and ten strokes will transfer 99.9 per cent to the sample tube.

A practical application of a Toepler pump constructed in this way is described in Chapter X and Fig. 64 where it is noted that, by calibrating the tube below the stopcock in the same way as has been described for tube *C* of a McLeod gauge, the volume of gas transferred can be measured. This is done by setting the instrument at each stroke as for a McLeod gauge and noting the



reading before opening the stopcock and compressing the gas through it.

Toepler pumps of various patterns and due to various authors are described later in the chapters concerned with deuterium and tritium. They all work on the principle described above, but not all of them could be calibrated for quantitative work.

### *Mercury Cut-offs*

Volatile materials such as carbon disulphide which attack stopcock grease are most conveniently stored *in vacuo* behind a mercury cut-off, and there will consequently be several of them on the high-vacuum apparatus. Textbooks on high-vacuum technique (7, 8) usually illustrate several designs, but in the present author's opinion that illustrated in Fig. 13 is the most convenient. It is small, relatively easy to make, and requires little mercury. Its construction involves only straightforward glassblowing except for the mercury float valves. These are constructed and tested separately before being assembled into the cut-off.

*Valves.* A piece of 10-mm. tubing is constricted at about its mid-point to about half its original diameter and slightly thickened, care being taken to make it symmetrical and coaxial on both sides of the constriction. The floats may be made either of glass (as illustrated) or, if gases and vapors which do not attack steel are the only ones which will be brought in contact with them, of steel, in which case  $\frac{1}{4}$ -in. ball bearings may conveniently be used. To make glass floats, a rod of about 8 mm. diameter and of a convenient length to handle is drawn to a taper at one end in the blowpipe. It is then ground gently into the constricted part of the tube with Carborundum paste until a neat ring of ground surface about 2 mm. wide is formed. If the ring of ground glass is not of uniform width, it indicates that either float or socket is misshapen and will probably leak. When grinding is complete, both parts are thoroughly washed, the float cut down to about 2.5 cm., and the cut end rounded in the flame.

If a ball bearing is to be used for the float it is possible to grind in several seatings with one bearing mounted with wax in the

end of a length of glass tubing. As so much more glass than steel is removed by the grinding, several seatings may be ground with a single bearing and a new one chosen to form each float.

The valve is tested by inserting the float in the tube, clamping vertically in a beaker of clean mercury, and applying vacuum.

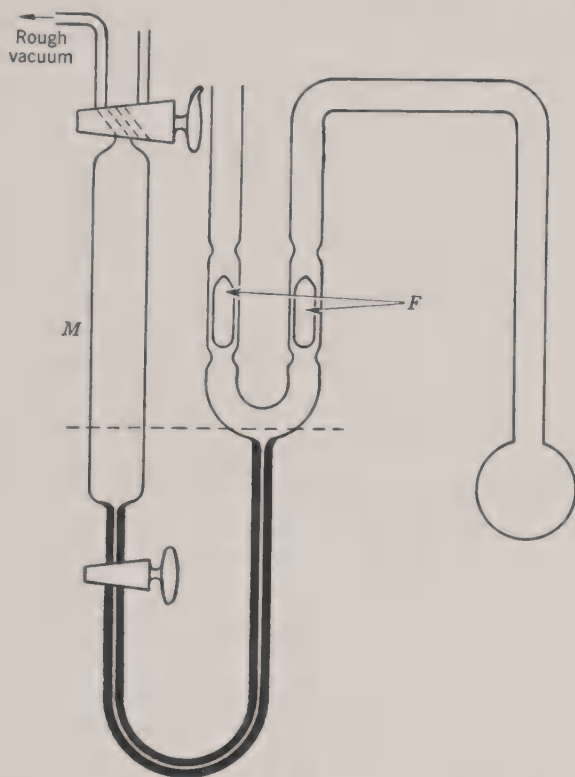


FIG. 13. Mercury cut-off. Diagrammatic view to show construction. In practice the device is built with the mercury reservoir *M* in front of the valves. The thick-walled capillary tube is therefore in a plane perpendicular to the page. Dotted line shows level of mercury when cut-off is open. *F* = floats.

The rising mercury should drive the valve firmly into its seating but should not leak past it. Leakage may be due not only to bad construction or grinding but also to moisture or dirt. It is most important that the glass surfaces should be clean and dry and the mercury freshly filtered.

When two mercury-tight valves have been made, they are connected together in the form of a U as shown in Fig. 13, pips being made in the glass tubing to support the floats about 1 cm. below their closed position. The two valves are then connected by means of thick-walled tubing to a mercury reservoir bearing a capillary stopcock at its lower end and a two-way stopcock at its upper end. The dimensions of the mercury reservoir and the volume of mercury which will be charged into it must satisfy two requirements. Firstly, when the mercury is driven into the valves to close them some must still remain in the reservoir above the capillary stopcock; secondly, when the mercury stands at the same height in both main limbs of the instrument it must leave a free passage between the two valves.

The cut-off is assembled into the vacuum system, empty of mercury, by first sealing the left-hand valve into the working line or manometric chamber (Fig. 1) and then sealing one arm of the two-way stopcock into the rough vacuum line. The whole instrument is then evacuated on the main vacuum pumps and warmed with a soft flame to eliminate adsorbed gas and moisture and charged with mercury. Then, if air is admitted to the reservoir and the capillary stopcock carefully opened, mercury will rise in the valves, driving the floats home with a slight click and isolating the solvent bulb. By evacuating through the two-way stopcock with the rough vacuum pump and opening the capillary stopcock, the mercury will be withdrawn from the valves, leaving the solvent bulb and main line once more in communication.

Once it has been ascertained that the valves are working properly, the solvent may be introduced into the bulb by distillation *in vacuo*. Once this has been done it will not be possible to open the valve furthest from the bulb unless the vapor pressure of the solvent is first reduced by cooling in liquid air or solid carbon dioxide. Then, as the solvent warms up and its vapor pressure rises, the desired volume of vapor may be taken. The excess is condensed back into the bulb by cooling again and the valves closed.

Mercury cut-offs, properly constructed and installed, give years of trouble-free service. In some circumstances, for example

where a supply of vapor will be needed over a relatively short period, they may be replaced by stopcocks lubricated with silicone grease. This is much less soluble than is ordinary high-vacuum grease in many solvents, including carbon disulphide, but, in the author's experience, leakage will occur after a few weeks unless the stopcock is regreased at frequent intervals.

## REFERENCES

1. R. C. ANDERSON, Y. DELABARRE, and A. A. BOTHNER-BY, *Anal. Chem.* **24**, 1298 (1952).
2. R. F. GLASCOCK, *Biochem. J. (London)* **52**, 699 (1952).
3. C. V. ROBINSON, *Rev. Sci. Instr.* **22**, 353 (1951).
4. D. L. BUCHANAN and A. NAKAO, *J. Am. Chem. Soc.* **74**, 2389 (1952).
5. D. D. VAN SLYKE and J. FOLCH, *J. Biol. Chem.* **136**, 509 (1940).
6. J. J. NAUGHTON and M. M. FRODYMA, *Anal. Chem.* **22**, 711 (1950).
7. ARNOLD L. REIMANN, "Vacuum Technique," Chapman and Hall, London, 1952.
8. A. FARKAS and H. W. MELVILLE, "Experimental Methods in Gas Reactions," Macmillan, London, 1939.



## CHAPTER III

# GAS PHASE ASSAY OF CARBON-14

## Discharge Tubes

### ELEMENTARY THEORY

When a nuclear particle enters a gas from an external radioactive source, or is generated within it because the gas itself is radioactive, ionization is produced. The number of ion pairs depends on the nature and energy of the particle; a 1-Mev beta particle in air at N.T.P. produces about 50 ion pairs per centimeter of path, whereas an alpha particle of the same energy produces about  $10^4$  ion pairs per centimeter. The gas discharge tube, whether functioning as an ionization chamber, proportional counter, or Geiger counter, depends for the measurement of radioactivity on this ionization and the effect on it of an applied electric field.

The commonest type of gas discharge tube consists of a conducting cylinder down the middle of which, and insulated from it, passes a wire. The tube contains gas, usually at a pressure less than atmospheric but not necessarily so, and there is a potential difference across the cylinder and wire which usually form the cathode and anode, respectively. If an ionizing particle enters the tube, the result will depend on the magnitude of the potential difference. This is shown diagrammatically in Fig. 14 where the charge collected on the anode is plotted against the potential difference between it and the cathode.

At all values of the applied potential there will be an attraction of electrons towards the center wire (+) and of positively charged gas molecules towards the cylinder (-). At low values, however, the charge collected will be less than the charge generated in the initial ionizing event because of recombination of

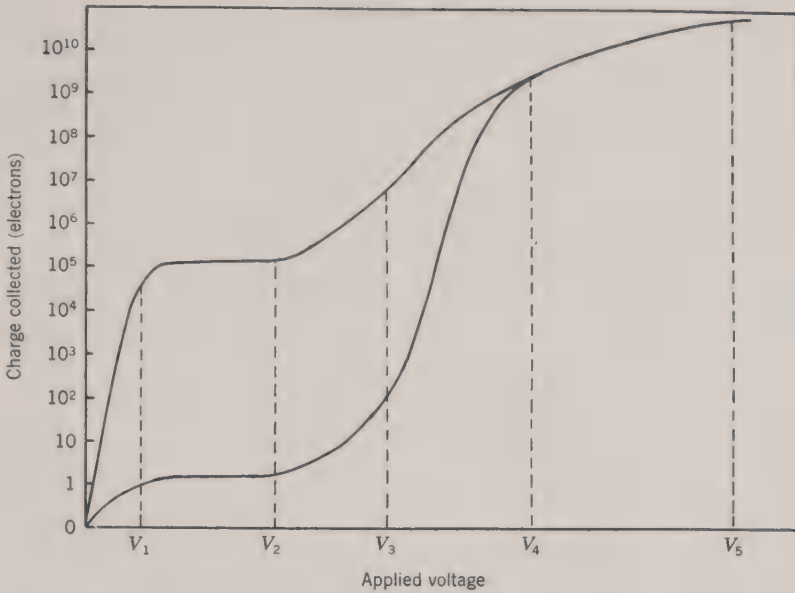


FIG. 14. Voltage and charge collected. The initial ionization is set in this example at  $e$  and  $10^5 e$  and the charge collected plotted against the voltage between the electrodes of a discharge tube. (After C. G. and D. D. Montgomery.)

$O-V_1$ : Region where recombination of ion pairs occurs.

$V_1-V_2$ : Region of *saturation*, where all charge produced is collected.

$V_2-V_3$ : *Proportional region*, where the charge collected is greater than that originally produced by the gas amplification factor  $A$ .

$V_3-V_4$ : Region of limited proportionality where  $A$  ceases to be constant at any given voltage.

$V_4-V_5$ : Geiger region, where charge collected is independent of initial ionization; same charge collected from an initial ionization of  $10^5 e$  as from one of  $e$ .

ion pairs. This corresponds to the region  $O-V_1$  in Fig. 14. As the voltage is further raised, all the ions generated by the ionizing particle are collected. This is the range  $V_1-V_2$  and is the region of operation of ionization chambers. At still higher voltages the ions, particularly the extremely mobile electrons, are sufficiently accelerated to cause further ionizations by collision. The sec-

ondary electrons thus formed are themselves accelerated towards the anode and cause further ionization, the whole cumulative effect being known as the Townsend avalanche, after a pioneer in this field. The change in potential of the electrode may be as much as 100 volts. This *gas amplification*, the result of ion multiplication by collision, denoted by the symbol  $A$ , is the important feature of true counters as distinct from ionization chambers and may have values up to  $10^9$  depending on conditions. This is to say, the charge collected may be up to  $10^9$  times as great as that produced in the gas by the original ionizing particle.

At any given voltage in the region  $V_2$ - $V_3$ ,  $A$  is substantially constant and the charge collected is proportional to the number of ion pairs first produced. For that reason it is known as the *proportional region*. Between  $V_3$  and  $V_4$ ,  $A$  is not constant at any one voltage but depends also on the number of ion pairs formed. It is called the region of *limited proportionality*. Beyond  $V_4$ , the *Geiger threshold*, the charge collected is independent of the number of ion pairs first produced and a single electron of any energy can start the discharge and produce a pulse of the same size as that which would be produced by a much more intensely ionizing particle such as an alpha particle. Counters may be operated in both the proportional and the Geiger regions according to the nature of the counting problem and the equipment used.

### IONIZATION CHAMBERS

No gas amplification takes place in ionization chambers, and the voltage applied need be sufficient only to saturate the gas, i.e., to ensure the collection of all ions. Ionization chambers have no dead time during which response to an ionizing particle is not possible and, in this respect, are superior to counters since these are insensitive during the period of time occupied by the discharge. Ionization chambers are therefore particularly useful for the measurement of very high activities since there is virtually no limit to the ionization current that can be measured. By the use of suitably designed equipment, however, a chamber may be operated so that as little as 30 disintegrations a second can be detected. The problem to be solved in the measurement of such

low activities is the measurement of ion currents of the order of  $10^{-15}$  amps (1) which necessitates specially designed direct-current amplifiers. A discussion of the electronic circuits by means of which this is accomplished is beyond the scope of this book but an account will be found in several standard works and in Taylor's monograph on the measurement of radioisotopes (1). An activity of 30 particles a second corresponds to a saturation current of about  $2 \times 10^{-15}$  amps. Such currents may be most reliably

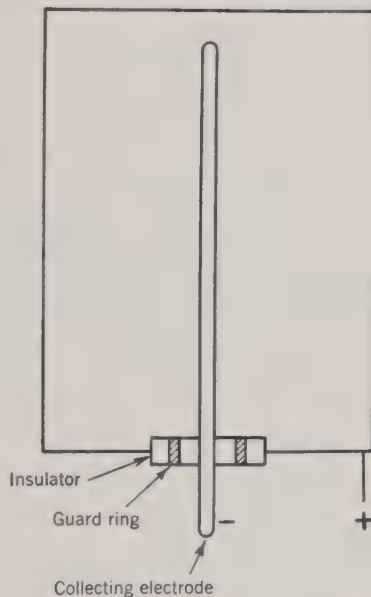


FIG. 15. Ionization chamber, showing essentials.

measured by means of the vibrating-reed electrometer, which is now superseding other forms of direct-current amplifier.

In its simplest form the ionization chamber consists of a metal cylinder with a center electrode well insulated from it as shown in Fig. 15. The most important feature in the construction is the insulator since any current leaking across it or produced in it will be measured and recorded as ionization current. According to Brownell and Lockhart (2), the difficulty lies not so much in obtaining a material of sufficiently high resistance but in eliminating stress currents which are generated in it both by mechanical

5751  
LIBRARY  
26-2-64



and electrical stresses. When the chamber is evacuated prior to filling it with radioactive gas, the mechanical stresses caused by the evacuation may give rise to currents large enough to introduce an appreciable error into the measurements. These workers therefore recommend that at least 30 minutes should elapse between filling the chamber and taking any measurements.

The center electrode is not made of wire as it is in proportional and Geiger counters since a thin wire can cause high local field strengths with consequent acceleration and multiplication of ions by collision. This gas amplification, which is exploited in counters, is to be avoided as a source of error in ionization chambers. The center electrode is therefore a rod several millimeters in diameter and, as a further precaution against ion multiplication, the cylinder is usually made positive, thus collecting the more mobile electrons on a large surface. The guard ring in the insulator is another important feature since it shields the center electrode from low-frequency radiation which is not intercepted by the cylinder and which it would otherwise pick up. It may be grounded or operated at the same potential as the center electrode, thus protecting it from leakage currents.

Unlike Geiger counters, which are operated at pressures well below atmospheric, ionization chambers can be operated at atmospheric pressure or above since, if the particles are very energetic, it is obviously desirable to introduce sufficient gas in their path to stop them and thus bring about the maximum possible ionization. For the counting of weak  $\beta$ -emitters such as  $C^{14}$  and tritium, however, high pressures are not required.

Ultimately, the success or failure of an ionization chamber as an instrument for the routine measurement of gas activities depends on its being accurately constructed from suitable materials. The cylinder metal must be as free as possible from alpha-active impurities since alpha particles are intensely ionizing and will cause high background currents. The whole instrument, and particularly the insulator, must be most carefully cleaned because electrical leakage is most likely to occur across dirty surfaces.

It will be concluded from the above description that the

routine operation of an ionization chamber for the accurate determinations of low or moderate activities is not an easy matter. Firstly, there is the necessity, already noted, of waiting for stress currents to die away, and this may be intolerable in a busy laboratory. Secondly, the instrument does not measure disintegrations directly and must be calibrated against a standard source of activity which itself will usually have been standardized by a counting technique. Thirdly, the recording instruments, notably the vibrating-reed electrometer, are very expensive and at present (1953) cost considerably more than the recording equipment used with counters.

On the other hand, however, it should be noted that ion chambers will hold more gas than the average gas counter, that of Brownell and Lockhart holding about 250 ml. (11 millimoles). They are also very useful for the measurement of very high activities and can be applied, for example, to the assay of very active tritium samples without the occurrence of the troublesome memory effects that are observed when tritium is subjected to the Geiger discharge (3). Direct-current amplifiers are also inherently simpler than counting equipment and this implies a greater degree of reliability.

### Measurement of $C^{14}O_2$ in Ionization Chambers

#### *Method of Brownell and Lockhart*

The design of ionization chamber used by these workers (2) is illustrated in Fig. 16 and is based on that of Borkowski of the Oak Ridge National Laboratory where it is used routinely. It has a capacity of 250 ml. and is made of brass with a mirror finish on all internal surfaces. The insulator is of molded polystyrene. A low alpha background is achieved not only by choosing a brass of low natural activity and carefully cleaning all surfaces but also by the application of a heavy coat of silver paint (No. 4817, E. I. du Pont de Nemours and Co. Inc.).

The diagram of the filling apparatus recommended for use with these chambers is taken from Brownell and Lockhart's paper and is shown in Fig. 17. It provides for the filling of chambers

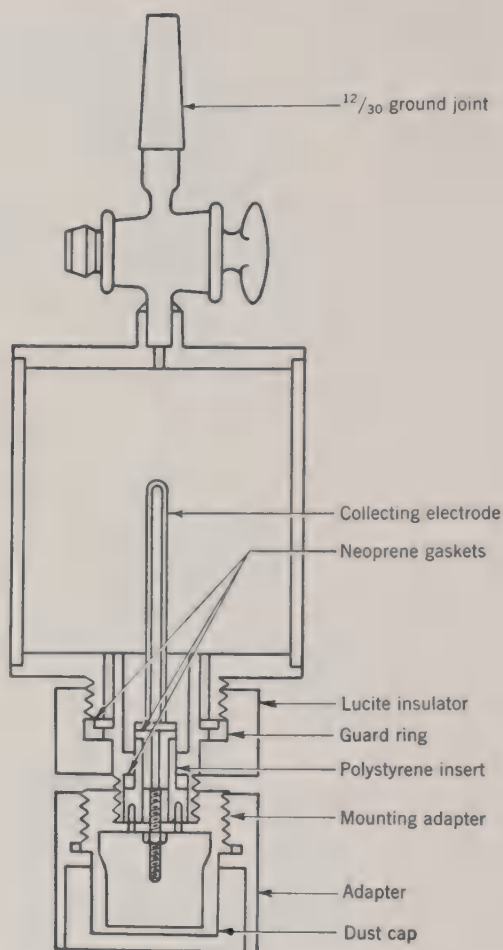


FIG. 16. Ionization chamber (Borkowski), as used by Brownell and Lockhart. This chamber has an internal volume of about 250 ml. and thus holds about 11 mM of  $C^{14}O_2$  or tritium at atmospheric pressure. It is sensitive to about 100 disintegrations/min. of  $C^{14}$  and about 10 times as much tritium because of the lower average energy of tritium beta particles.

with carbon dioxide obtained from barium carbonate which, in a biochemical experiment, would presumably have been previously prepared by the absorption of combustion carbon dioxide in barium hydroxide solution. The ion trap shown on the extreme left-hand side of the diagram is provided for the removal of heavy organic molecules and ions which, according to these workers, are

present as an impurity in commercial carbon dioxide and which would cause a spurious current in the ionization chamber.

The whole system is evacuated through stopcock 10 by a mercury diffusion pump backed in the usual way with a mechanical pump. The radioactive barium carbonate is placed in the reaction vessel *B* which is attached to the apparatus by means of the standard taper joint lubricated with silicone grease. The reaction vessel and spiral trap *C* are evacuated, stopcock 3 is closed, and concentrated sulphuric acid is dripped into *B* until no more carbon dioxide is evolved. Spiral trap *C* is then chilled in liquid nitrogen and the carbon dioxide allowed to condense into it by

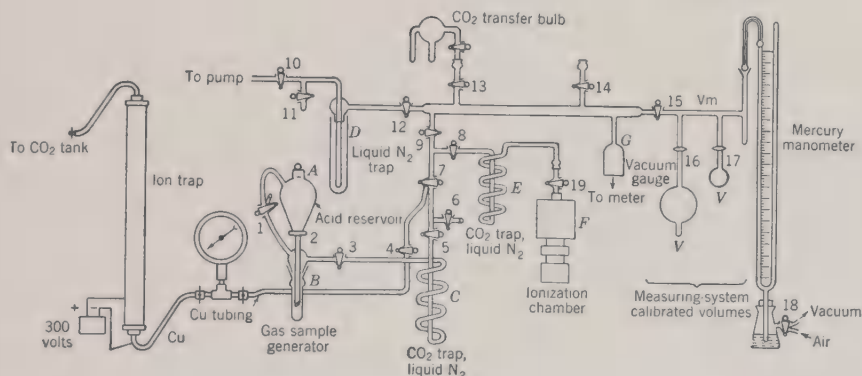


FIG. 17. Filling apparatus and gas transfer line (Brownell and Lockhart).

slowly opening stopcock 3. The volume of gas can be measured if desired by means of the manometer and the calibrated volumes *V* and *v<sub>m</sub>*.

No provision is made for actually condensing a measured volume of carbon dioxide into the ionization chamber *F* and in this respect the method seems less convenient than, for example, the Geiger counting method of Brown and Miller as used by the present author (Chapter IV). Brownell and Lockhart offer a choice of two methods for introducing a known volume of gas into the chamber. If the volume is small, the carbon dioxide is condensed into the spiral *E* after first evacuating both trap and chamber. Stopcock 8 is closed and the condensed gas allowed to vaporize. Inactive carbon dioxide is then allowed to fill the main part of the apparatus through stopcock 4 until the reading on the



manometer indicates that the pressure is greater than that which the active sample is expected to exert in the chamber. Stopcocks 8 and 19 are opened and the active sample swept into the chamber by the onrush of gas. The pressure is quickly adjusted to 50 cm., the working pressure of the chamber, and stopcocks 4 and 19 are closed. The chamber is then removed from the filling apparatus at the standard joint and attached to the electrometer.

The other method is used when the available volume of radioactive carbon dioxide is large. The gas is liberated, transferred to the spiral trap *E*, and allowed to warm up and expand into the chamber. The fraction of the total sample actually contained in the chamber when stopcock 19 is closed is known from the previously determined capacity of chamber and trap. The active gas left in the trap is then pumped away and the pressure in the chamber adjusted to 50 cm. with inactive gas from the reservoir.

*Measurement of activity.* The chamber charged with radioactive carbon dioxide is placed on the head of a vibrating-reed electrometer (Argonne National Laboratory Model) and fixed with three screws. The auxiliary apparatus consists of a recorder and a 300-volt battery whose positive pole is connected to the cylinder of the chamber and whose negative pole is connected to the head of the electrometer. At least 30 min. is allowed to elapse for stress currents to die away, and the apparatus is then switched on. The ionization current is reflected in the rate of drift of a millivoltmeter needle or, more conveniently, on an automatic chart recorder plotting voltage changes. This may be calibrated using a standard source of radiocarbon dioxide so that the activity corresponding to a measured rate of drift can be calculated.

#### *Electroscope Method of Henriques and Margnetti*

These workers (4) have described an ingenious adaptation of ionization chamber and Lauritsen electroscope to the measurement of radiocarbon activity. The apparatus, shown in Fig. 18, consists essentially of a 200-ml. quartz bulb completely silvered on the inside except for a small area through which the collecting electrode passes. The silvered surface of the bulb forms the anode and the wire, which is connected to the gilded quartz fiber of the

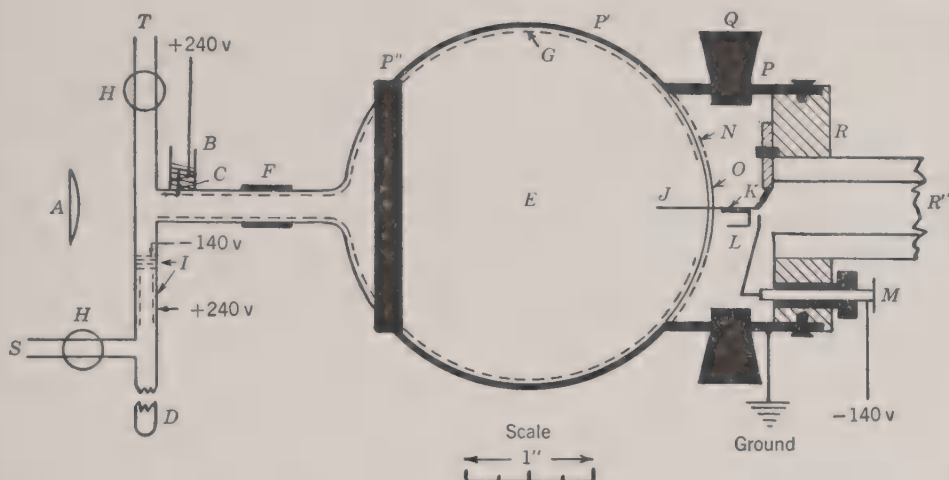


FIG. 18. Ionization chamber-Lauritsen electroscope (Henriques and Margnetti).

A: Light source 6 watts) for illumination of quartz fiber and scale.  
 B: Mercury well.  
 C: Tungsten wire, providing electrical connection to silvered surface.  
 D: Condensing tube.  
 E: Quartz flask-ionization chamber.  
 F: Pyrex-quartz graded seal.  
 G: Silvered portion of flask.  
 H: High-vacuum pressure stopcocks.  
 I: Ion trap (very coarse, silvered, fritted disk and silvered glass tube).  
 J: Ion-collecting wire (3-mil tungsten).

K: Fiber repelling post.  
 L: Gold plated quartz fiber.  
 M: Electroscope charge button.  
 N: Portion of flask coated with aquadag.  
 O: Thin coat of ceresin wax.  
 P: Brass adaptor.  
 P': Brass straps, 0.25 in. (4 in all).  
 P'': Brass collar provided with screw for clinching straps.  
 Q: Rubber-stoppered hole, 0.375 in. in brass adaptor (4 in all).  
 R: Lauritsen electroscope frame.  
 R': Electroscope tube containing objective, scale, and eyepiece.  
 S: To vacuum system.  
 T: To mercury vapor pump.

electroscope, forms the cathode. The radiocarbon dioxide sample to be assayed is introduced into the bulb and the pressure adjusted to 2 atmospheres. The silvered surface is maintained at a constant positive potential of 240 volts and the quartz fiber charged to a potential of -140 volts. Positive ions are collected on the cathode thus making the quartz fiber and repelling-post

less negative, with the result that the fiber moves towards the repelling-post under the restoring force of its own elasticity. The rate of motion of the fiber, which is observed against an eyepiece scale in a microscope attached to the instrument, is a measure of the activity of the gas sample. The method is very sensitive, allowing activities of about  $5 \times 10^{-5} \mu\text{C.}$  in the whole 20 millimoles of gas to be measured to within  $\pm 2\%$ , but it suffers from the serious disadvantage that two to three hours are required for such a determination.

### COUNTING TUBES

The first counting tube to make use of gas amplification was constructed by Rutherford and Geiger in 1908. It consisted of a brass cylinder about 1.8 cm. in diameter and 25 cm. long which

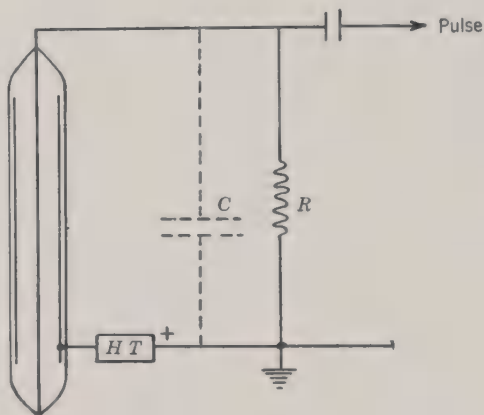


FIG. 19. Fundamental counter circuit.

was sealed at each end with ebonite plugs through which a coaxial wire passed. The air pressure in the cylinder was reduced to a few centimeters of mercury and a potential difference of about 1000 volts was applied through an electrometer to the electrodes, the center wire being positively charged. Rutherford and Geiger found that, when alpha particles were admitted to a tube in this condition, the arrival of each particle was indicated by a pulse on the electrometer. Since 1908 the most important modifications of Rutherford and Geiger's first counter have been in the circuits and devices used for operating the counter and recording its signals and in the nature of the gaseous filling.

The fundamental counter circuit is shown in Fig. 19. Early workers found that unless the resistance  $R$  was very large ( $10^9$  ohms, or more) the counter would not function but would give a continuous pulse discharge. The quenching of the discharge by means of such a high anode resistance, however, resulted in a high time constant for the circuit, with the result that the time elapsing after each pulse before the counter could respond to another

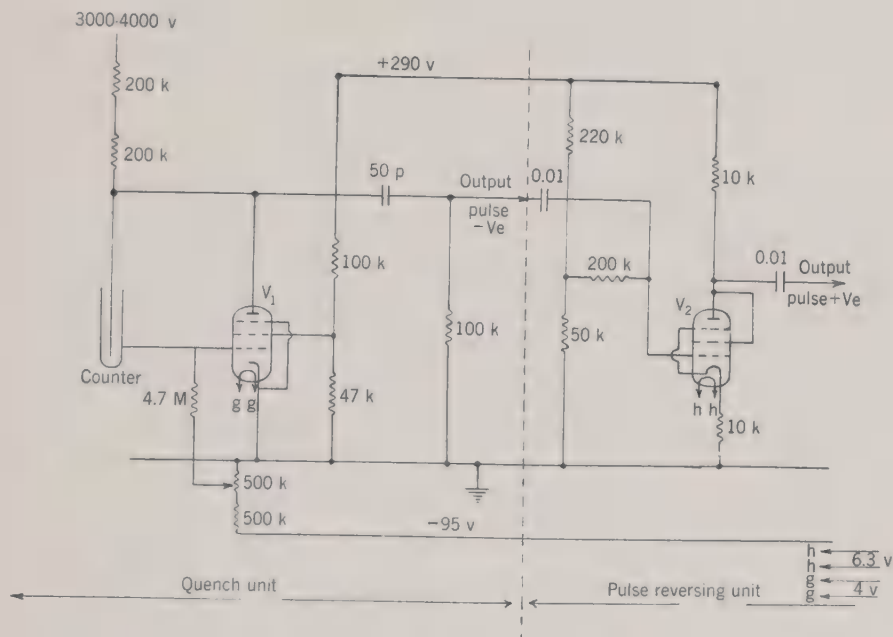


FIG. 20. Neher-Harper type quench circuit with pulse reversing unit, thus giving a positive pulse.  $V_1$  = MS/Pen T, tested to maximum peak anode voltage of 4 kv.  $V_2$  = SP61.

ionizing particle was too long for fast counting. Electronic quenching, which became possible with the invention of the vacuum tube, permitted the construction of counter circuits with short time constants and, therefore, suitable for fast counting. A quench circuit such as that devised by Neher and Harper (5) has proved very satisfactory. It works on the principle that the *positive* pulse arriving at the cylinder is transmitted to the grid of a vacuum tube, thus making the tube conducting and short-circuiting the counter. (See Fig. 20.) Such circuits are still useful



for special purposes, such as the gas counting of  $C^{14}$ , notwithstanding the invention of self-quenching counters.

In 1937, Trost (6) discovered that counters containing the usual gas mixed with a small proportion of a polyatomic vapor such as alcohol were self-quenching and each ionizing particle entering the counter was recorded as a single pulse. This permitted counting without a quench circuit and with an anode resistance as low as  $10^6$  ohms. Since a low anode resistance means a low time constant for the circuit, fast counting thus became possible and self-quenching counters are still sometimes known as "fast counters." The mechanism of quenching in a tube containing an organic vapor now seems fairly well established. The neutralization of positive ions by electrons at the cathode results in the emission of electromagnetic radiation which, in the absence of a quenching vapor, causes the emission of secondary electrons from the walls of the counter, thus maintaining the discharge. This radiation is absorbed by vapor molecules, consisting of four atoms or more, without the production of secondary electrons. The absorption of radiation causes the dissociation of the organic vapor which gradually becomes used up. A self-quenching Geiger counter operating at a gas amplification of about  $10^7$  becomes useless after about  $10^9$  discharges owing to depletion of the quenching agent. A proportional tube operating at a gas amplification of anything above  $10^2$  needs a quenching agent to maintain stability but has a life of at least  $10^{12}$  discharges owing to a much lower rate of decomposition of the quenching agent. These considerations are not normally of any importance in gas counting, however, since a new filling is usually put into the counter each time it is used.

If a counter containing the appropriate gas mixture is exposed to a constant source of radiation, and the applied voltage raised gradually, no pulses will be recorded below a certain voltage known as the *starting* or *threshold voltage*. Then, as the voltage increases, the counting rate also increases until a plateau is reached the beginning of which is the *Geiger threshold*. Over the range of voltage corresponding to the plateau, i.e., the Geiger region, the counting rate is nearly independent of the voltage.

Beyond the Geiger region, the counting rate again rises rapidly with increasing voltage. In the region before the Geiger threshold lies the proportional region which may or may not show a plateau depending on the filling gas and the detecting equipment used.

A plot of counting rate against applied voltage, such as that illustrated in Fig. 21, is known as the *characteristic* of the counter, the actual voltages corresponding to the various regions referred to depending on the size and geometry of the counter and the

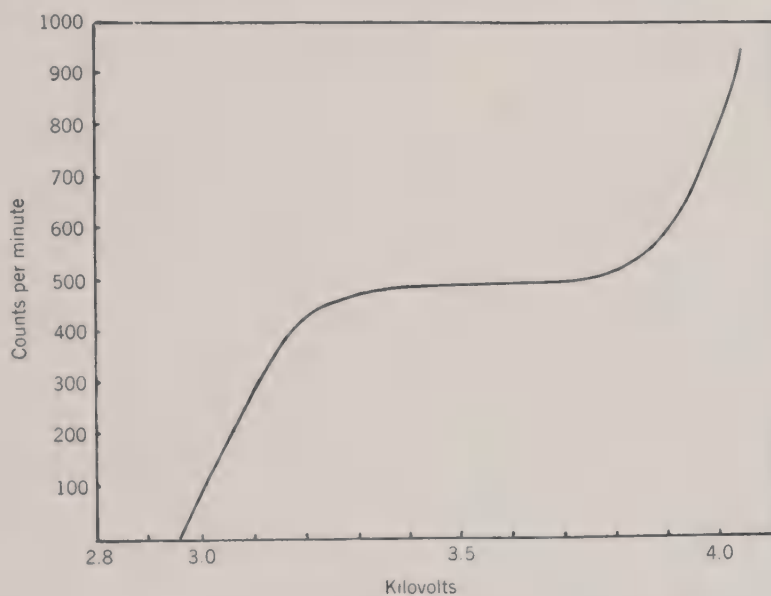


FIG. 21. Counter characteristic. Plotted for a graphite-cathode counter filled with  $\text{CO}_2$  at 20 cm. and  $\text{CS}_2$  at 2 cm. pressure.

nature and pressure of the filling gas. A good proportional or Geiger counter must have a plateau to be of any practical use otherwise very precise voltage stabilization must be used. This plateau should extend over at least 200 volts with a slope of not more than 3% per 100 volts.

The commercially available equipment commonly used for Geiger counting consists of a high-voltage unit (E.H.T.), giving voltages up to 3 or 4 kv, a scaling unit for recording the pulses, and a quench circuit or preamplifier, according to whether the counter is self-quenching or not. A proportional counter will need

in addition an amplifier with a gain of at least 1000 and a discriminator for the elimination of very small pulses due to noise and pickup.

### *Gas Counters; General*

*Shape.* Gas counters are nearly always cylindrical, although not necessarily so. Bernstein and Ballentine (7) describe a proportional counter with spherical geometry which gave a satisfactory performance. The conventional gas counter, however, whether intended for Geiger or proportional counting, usually consists of a glass tube carrying a stopcock and standard joint and containing the cylinder and anode wire within it. The glass is usually Pyrex and the electrical connections from outside to inside are therefore made through tungsten-to-glass seals.

*Electrodes.* The anode wire is preferably of tungsten since it is strong, chemically inert, readily sealed to Pyrex glass, and will stand being heated electrically to red heat, if necessary, after the counter has been evacuated. Generally speaking, the thinner the wire the lower the operating voltage, and wires from 0.002 to 0.004 in. are commonly used. The wire must be smooth and free from sharp points to avoid high local fields and point discharges. It is usual also to protect the wire with glass sleeves where it is welded to the thicker tungsten supports. These reduce the likelihood of spurious pulses due to irregularities in the weld and also help to prevent electrical leakage to the cathode across the intervening glass surface (see Fig. 22).

The cathode may be either a metal-foil cylinder, fitting snugly against the inside of the glass envelope, or a conducting film deposited on it. Bernstein and Ballentine recommend glass counters with chemically deposited silver cathodes for proportional counting of  $\text{CO}_2$  but state that evaporated chromium or gold may also be used. Brown and Miller (8), whose admirable method of counting  $\text{CO}_2$  in the Geiger region is used by the present author as a routine measure, prepare their cathodes by silvering the glass and then coating with colloidal graphite to reduce secondary electron emission. The present author has found, however, that counters made without preliminary silvering, and having

cathodes consisting only of a graphite film, are quite satisfactory and are available commercially.

*Size.* For successful operation, the size of counters may vary between fairly wide limits. Thus, Bernstein and Ballentine constructed proportional counters with cathodes varying from 5 to

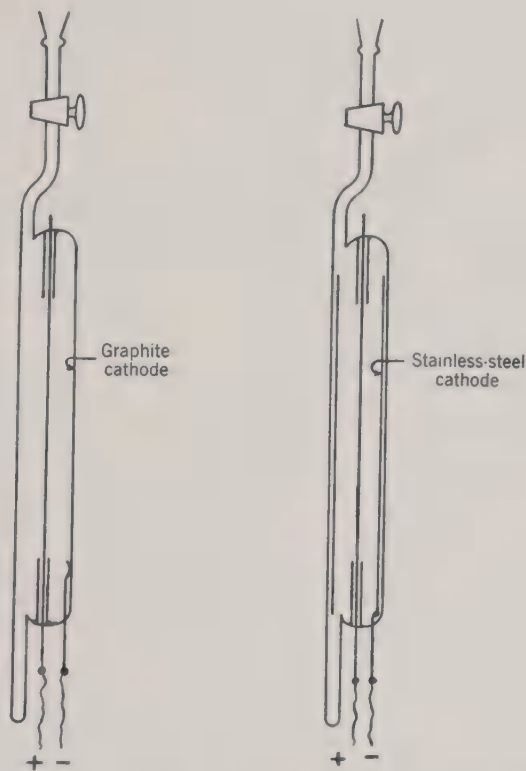


FIG. 22. Gas counters. Two patterns recommended by the author. Both are of outside diameter 27–30 mm. and tube length of 16 cm. Total length, including stopcock, standard joint, and condensing tube, is 35 cm.

20 cm. in diameter and from 10 to 100 cm. in length. All had good characteristics. Brown and Miller constructed satisfactory Geiger counters varying between 1 and 4 cm. in diameter, 9 and 28 cm. in length, and 13 and 300 ml. in internal volume. It must be remembered, however, that the operating voltage, which depends both on gas pressure and cathode diameter, must be kept to a reasonable value and certainly within the range of the high-



voltage equipment. Furthermore, the tube should not be unwieldy and should be of such a length that, when on the filling manifold, there is room for a Dewar vessel of liquid air to be placed beneath it for the purpose of condensing in the filling. Obviously it should not be very heavy, otherwise it will not be possible to attach it to the high-vacuum apparatus by means of its standard joint and hooks only. These considerations place an upper limit on size. The lower limit is placed by the consideration of decreased efficiency resulting from a decreased sensitive volume-total volume ratio which is inevitable in small counters and, more important, by the limit to the total volume of radioactive gas that can be counted. In short, a counter should be as large as it is convenient to handle and operate on the available electronic and high-vacuum equipment. The standard proportional counter used by Bernstein and Ballentine was 2 cm. in diameter, 30 cm. long, and, therefore, having an internal volume of just over 90 ml. Eidinoff (9), using the Brown and Miller method of Geiger counting, used a standard counter of 1.55 cm. internal diameter, 15–16 cm. cathode length, and an internal volume of about 30 ml. The present author, using the Brown and Miller method, has found that counter failure, due apparently to electrical leakage across the glass between the electrodes, frequently occurs with counters of diameter as small as 1.5 cm. at the voltage of about 3000 volts necessitated by a  $\text{CO}_2$  pressure of 20 cm. He therefore recommends counters at least 2.5 cm. in internal diameter and 15–16 cm. long. These tubes have an internal volume of about 80 ml. and, when fitted with the usual stopcock, standard joint, and condensing tube, have a total length of about 35 cm., which enables them to be attached comfortably to the high-vacuum apparatus described in Chapter II.

### *Proportional Gas Counters*

*Characteristics.* Proportional counters are operated, as already noted, in the region  $V_2$ - $V_3$  of Fig. 14 where the charge collected is proportional to the amount of ionization produced in the initial ionizing event. Proportional counting has the advantage over Geiger counting that the dead time of the counter is

very short. This permits the counting of much more active samples than is possible in the Geiger region. Thus Bernstein and Ballentine (7) found no loss of counts at rates of 200,000 per minute. This is an activity some 50 to 100 times greater than can be accurately measured in the Geiger region and means that the dead time must have been 3  $\mu$ sec. or less. Such fast counting may, however, be of no advantage in biological work where low activities are more often encountered than high. It must also be noted, however, that the proportional region is more tolerant than is the Geiger region of impurities in the gas. White, Campbell, and Payne (10) found that although trito-methane, prepared by the action of radioactive water on aluminum carbide, showed no Geiger region owing to the presence of impurities, it could nevertheless be counted in the proportional region.

Bernstein and Ballentine claim that proportional counters are also preferable to Geiger counters at low counting rates because the background is lower and there are no spurious counts arising in the detector. The background stated by them for a methane-filled lead-screened counter with a sensitive volume of about 100 ml., however, is 100 counts/min. which, in the present author's experience, is slightly higher than that of a carbon dioxide-carbon disulphide filled counter of similar size operating in the Geiger region. According to Bernstein and Ballentine, the spurious counts arising in the high-voltage supply and amplifier can be easily eliminated and do not, therefore, impair the accuracy of their method. Their claim that Geiger counting by the method of Brown and Miller (see later) using carbon dioxide-carbon disulphide filled counters compares unfavorably with theirs because the Brown and Miller technique involves the use of variable fillings, with consequent inaccuracies, seems to be based on a misunderstanding. Brown and Miller do not recommend variable fillings and, in the present author's experience, a Geiger counter operated with the filling gas at a fixed pressure gives excellently reproducible results. A disadvantage of proportional counting lies in the necessity for two extra units—the amplifier and discriminator—which means extra expense, especially if several sets of equipment are installed, and there are also more things to go

wrong. On the other hand, it must be allowed that proportional counting is considered by many to be very reliable and more accurate than Geiger counting.

### Proportional Counting of Carbon Dioxide

#### USING METHANE-CARBON DIOXIDE MIXTURES

##### *Method of Bernstein and Ballentine*

The general design of the counters used by these workers (7) is shown in Fig. 23. The standard counter is 2 cm. in diameter, 30 cm. long, and, thus, has an internal volume of about 100 ml. The cathode is chemically deposited silver and the anode is

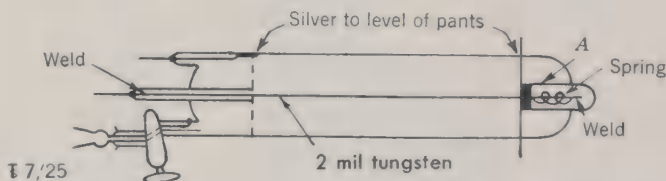


FIG. 23. Gas counter (Bernstein and Ballentine). For proportional counting of  $\text{CO}_2$ -methane mixtures.

0.002-in. tungsten wire. There is no condensing tube, but one end is kept free from glass-to-metal seals to permit immersion in liquid nitrogen. The electronic equipment consists of a scaler and a 5000-volt power supply. The scaler has an amplifier with a gain of 1000 and a discriminator with a maximum sensitivity of 1 volt. To overcome the difficulty of multiple pulses caused by overloading the amplifier with large input signals, a IN34 crystal diode, used to clip positive overshoots, was inserted at the input to the second loop.

The gaseous mixture used by these workers as a filling for their counters was carbon dioxide and methane at atmospheric pressure. For some reason that is not clearly explained in their paper they wished to recover the carbon dioxide from the counter "for later gravimetric analysis," which would not be possible if diluted with carrier carbon dioxide and the counter operated on that

gas alone. Thus the actual composition of their counting mixture was variable, depending on the amount of carbon dioxide being counted. They found that for a given activity the observed counting rate did not vary significantly with gas composition until the partial pressure of carbon dioxide was above 100 mm., when some losses of counts were noticed. This was apparently due to the raising of the threshold of the proportional region and to the steepening of the slope of the characteristic. Bernstein and Bal-

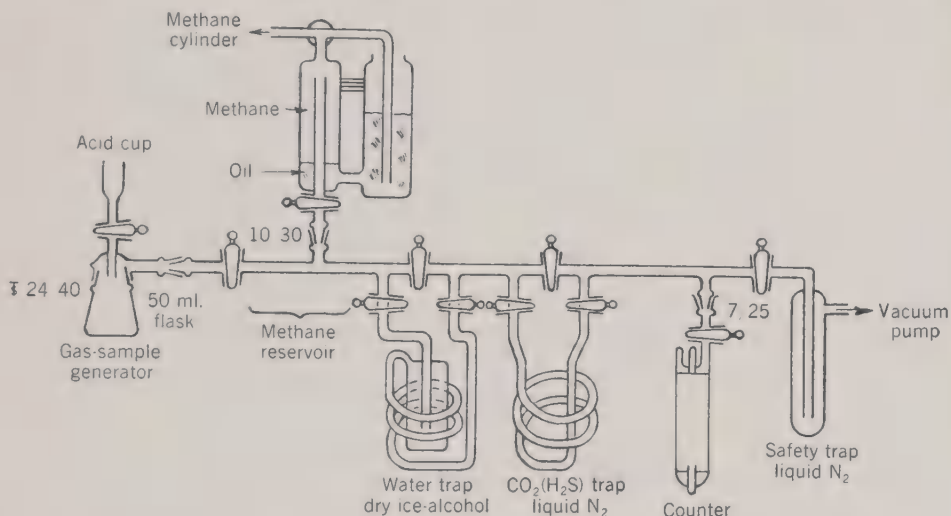


FIG. 24. Counter-filling manifold (Bernstein and Ballentine). Radioactive barium carbonate is decomposed, and the carbon dioxide is dried by means of a trap cooled to  $-78^{\circ}$  and condensed into another trap cooled with liquid nitrogen. It is then condensed into the counter and the pressure adjusted to atmospheric with methane from the reservoir.

lentine therefore recommend that the partial pressure of carbon dioxide should not exceed 100 mm. It is then possible to operate the counter with a fixed setting of the operating voltage and amplifier gain without the introduction of appreciable errors due to variations in the shape of the characteristic. At this partial pressure, of course, the maximum amount of carbon dioxide that can be counted in their standard counter is about 13 standard ml.

Figure 24 shows the manifold used for filling their counters. Radioactive barium carbonate is decomposed with acid, passed through a trap cooled to  $-78^{\circ}$  to remove water vapor, and then



condensed at liquid-nitrogen temperature so that any incondensable gas may be pumped away. Only a mechanical pump is used since pressures of 0.01–0.1 mm. mercury are sufficiently low for the work described; the measurement and counting of very small volumes of gas (10 to 100  $\mu$ l.) is not provided for. Finally, the sample is condensed into the counter and allowed to warm to room temperature. The pressure is then adjusted to 1 atmosphere with tank methane, and the counter is connected to the electronic equipment.

It is of interest to note that Van Slyke, Steele, and Plazin (11), whose wet combustion method is described in Chapter V, use the Bernstein and Ballentine method of proportional counting but make provision for counting at partial pressures of carbon dioxide greater than 100 mm. They do this by plotting a curve of efficiency against partial pressure and find that at 80–116 mm. the efficiency of their counter is 98%, falling smoothly and nearly linearly to 91.6% as the partial pressure rises to 260 mm.

#### USING CARBON DIOXIDE ALONE IN THE PROPORTIONAL COUNTER

##### *Method of Freedman and Anderson*

As the use of carbon dioxide-methane mixtures necessarily limits the size of sample that can be counted, the use of carbon dioxide alone would obviously increase the sensitivity of the assay method if, at the same time, the errors resulting from a steep plateau slope could be eliminated. Freedman and Anderson (12) found that the plateau slope of a carbon dioxide filled proportional counter was 10% per 100 volts, compared with 1% per 100 volts for a methane filled counter. A further limitation to the accuracy of counting low activities is imposed by the background. For maximum statistical accuracy the sample-to-background counting ratio must be a maximum. They attempted to solve the combined problems of plateau slope and sample-to-background counting ratio by the use of pulse-height discrimination. This was achieved with a differential discriminator with two independently adjustable "gates." All pulses greater than

one chosen size and less than another could thus be rejected and only a narrow region of the pulse-height spectrum actually counted. The authors point out that under such conditions "the usual voltage plateau is of no significance" although their reasons for this assertion are not quite clear. They point out further that pulse-height discrimination technique is most efficient in separating one monoenergetic radiation from another but that, in the proportional counting of low activities, the problem is the more difficult one of partly separating one spectrum (the sample) from another overlapping spectrum (the background) in the most efficient manner. It was found in practice that the optimum setting of the upper gate of the discriminator when counting radioactive carbon dioxide was 100; i.e., no upper gate at all. Hence it would appear that, although the sample-to-background ratio could be adjusted to a maximum value, the absence of an upper limit to the pulses being recorded would result in the counter being no different from any other proportional counter as far as its plateau slope is concerned and the problem of a 10% per 100 volts slope would remain.

*Method of Bradley, Holloway, and McFarlane*

These workers (13), operating their counters on carbon dioxide alone in the region of limited proportionality, achieve a markedly better characteristic than Freedman and Anderson who found that, with carbon dioxide alone, plateau slopes of 10% per 100 volts were observed. Bradley and co-workers claim for their method slopes not exceeding 4% per 100 volts.

Their counter is very similar in shape and size to that shown in Fig. 22, having a total tube length of 10 cm., an effective cathode length of 7.4 cm., and a tube diameter of 2 cm. The cathode is of graphite deposited directly on the glass. It differs from the counter illustrated in Fig. 22 in being designed to fit an upward-pointing cone on the vacuum apparatus. The condensation tube is therefore attached to the end nearest the stop-cock. The counter is operated at 20 cm. mercury pressure which is the lowest at which good counting properties are obtained.

The operating potential is in the region of 2600 volts and the pulses are fed into a linear amplifier and thence to a scaler. The total gain of the equipment is  $5 \times 10^4$  and the discriminator rejects all pulses below 25 volts.

This method of gas counting carbon dioxide is claimed by its originators to have several advantages over that of Brown and Miller (see Chapter IV) with which they chiefly compare it. Firstly it obviates the necessity for the carbon disulphide charge-transfer gas which they find difficult to manipulate in a vacuum system, and secondly it is thought to have a greater efficiency. They base this opinion on a report by Miller and co-workers (14) who, in measuring the half-life of  $C^{14}$  by means of both carbon dioxide-methane proportional counters and carbon dioxide-carbon disulphide Geiger counters, observed a discrepancy in their results which appeared to be explicable only on the assumption that the Geiger counter was about 15% less efficient than the proportional counter. This agrees with a finding of Bernstein and Ballentine (7) who observed that, for a given activity and a similar geometry, a Geiger counter gave a 13.9% lower count than did a proportional counter. On the other hand, Hawkings, Hunter, and Mann (15), after a very detailed study of the efficiency of carbon dioxide-carbon disulphide filled counters, reported a 97% efficiency for the sensitive zone. Bernstein and Ballentine suggest that the loss of counts in the Geiger counter used in their work (the counting was actually done by W. W. Miller, one of the originators of the method) may have been due to overquenching and, since Hawkings, Hunter, and Mann used a different kind of quenching unit, a Parkinson unit (16), this may in fact account for the conflicting results. Alternatively, the loss of counts in Bernstein and Ballentine's work may have been due to dead-time coincidence losses if the actual counting rate (which is not reported) was higher than about 2500 counts/min. In the present author's experience, however, the manipulation of carbon disulphide in a vacuum system presents no difficulties if the mercury cut-off described on page 44 (Fig. 13) is used and the slightly lower efficiency of the Geiger counting method—even if firmly established—does not offset its great convenience.



### Measurement of $C^{14}$ as Acetylene for Radiocarbon Dating

The natural radioactivity of contemporary carbon due to  $C^{14}$  has been estimated by Libby, Anderson, and Arnold (17) at 15 disintegrations/min./gm. of carbon. Owing to the decay of the isotope, the activity of carbon obtained from ancient samples of wood and other organic materials is measurably less than this and is nil in very ancient samples such as coal. Thus, as is now well known from the numerous papers published by Libby and his colleagues, a measurement of the natural radioactivity of carbon obtained from archeological samples provides a means of dating them. The chief difficulty in carrying out the measurement, however, is the low natural radioactivity quoted above and the consequent low counting rates to be expected, even in relatively efficient counting equipment. Libby and co-workers carry out the determination by combustion of the sample, reduction of the carbon dioxide with magnesium, and counting of elementary carbon in a screen-wall counter. The counting rate observed for modern carbon is then about 5 counts/min. in their equipment.

Because it is an inherently more efficient technique—and this is a problem in which efficiency is of more than usual importance—Barker (18) and Crathorn (19) use gas counting for the measurement of natural radiocarbon. Carbon dioxide, containing only one carbon atom per molecule is not, however, the obvious choice of counting gas in this case; a gas containing more than one carbon atom would be preferable but such gases are not readily prepared from inorganic carbon however, and Barker has chosen acetylene, for whose preparation from carbon dioxide there is a published method available (20). He considers that even a doubling of the mass of carbon in a unit volume of gas is worth the trouble of preparing it. Since it is desirable to operate the counter at as high a pressure as possible, thus introducing the maximum weight of carbon into the counting volume, the proportional region is used, and here again acetylene is preferable to carbon dioxide because counters filled with acetylene operate at a lower voltage than when filled with carbon dioxide at the same pressure.



### *Preparation of Acetylene (Method of Barker)*

The basis of the method is the combustion of the sample to carbon dioxide and reduction of the carbon dioxide to acetylene, using high-vacuum technique.

*Combustion.* This is carried out on the exceptionally large scale of 10–15 gm. of dry wood or coal (see Chapter V) because 3-liter counters filled with acetylene at atmospheric pressure are used for the actual counting. The combustion takes place in a large quartz tube which is not packed with the usual copper oxide filling because it has been found that so much heat is produced in this catalyst during a combustion on this scale that it attacks the quartz. Instead, the tube is filled with quartz chips and run at 900°.

The sample is heated gently in a stream of oxygen passing at the relatively low rate of 100 ml./min. This results in a slow, controllable initial combustion. More oxygen at a rate of about 200 ml./min. is injected into the heated zone of the tube, thus bringing about the complete oxidation of smoke and volatile substances. Finally, the combustion products are stripped from the gas stream by means of liquid-oxygen-chilled traps, and the carbon dioxide is separated from the water by sublimation at  $-78^{\circ}$  and stored in large bulbs.

*Conversion of carbon dioxide to acetylene.* This is carried out by the method of Arrol and Glascock (20) with certain modifications which arise from the use of a much larger scale. In Arrol and Glascock's method, carbon dioxide on the 0.05–0.5-ml. scale was allowed to react with barium metal in a stainless-steel vessel at 600°, yielding barium carbide which, on treatment with water, yielded acetylene in 95–100% yield. Barker uses lithium instead of barium because of its much lower equivalent weight and, working on the 6-liter scale with a much larger reaction vessel, obtains acetylene in about 65% yield. The yield is limited by the slow reaction between lithium carbide and water containing more than about 5% of lithium hydroxide in solution. This problem did not, of course, arise on the small scale used by the originators of the method.

## Counting

After purification, the acetylene thus produced is introduced into 3-liter counters made of mild steel as described by Crathorn (19). Mild steel is used because of its low natural radioactivity. The counter is surrounded by 11 Geiger counters arranged in anticoincidence and encased in a shield of lead 4 in. thick. It is operated in the proportional region and the discriminator is adjusted to give the optimum sample-to-background counting rate. The background is measured by using acetylene made from anthracite and is found to be 30 counts/min. When filled with acetylene made from modern carbon, the rate is 64.5 counts/min. Thus the whole range of natural activities from dead carbon to modern carbon is encompassed in a counting range of 34.5 counts. Assuming Libby's figure of 15.3 disintegrations/min./gm. to be correct, Crathorn observes that his value corresponds to a counter efficiency of about 75%. Higher efficiencies can be obtained with minimum discriminator setting, when much fewer  $C^{14}$  beta particle pulses are lost, but poorer sample statistics result. This efficiency is to be compared with that of screen-wall counters, which is usually about 5%.

## REFERENCES

1. D. TAYLOR, "The Measurement of Radioisotopes," Methuen's Monographs on Physical Subjects, London, 1951.
2. G. L. BROWNELL and H. S. LOCKHART, *Nucleonics* **10**, No. 2, 26 (1952).
3. W. J. ARROL and E. J. WILSON, private communication.
4. F. C. HENRIQUES, JR. and C. MARGNETTI, *Ind. Eng. Chem. Anal. Ed.* **18**, 417 (1946).
5. H. V. NEHER and W. W. HARPER, *Phys. Rev.* **49**, 940 (1936).
6. A. TROST, *Z. Physik.* **105**, 399 (1937).
7. W. BERNSTEIN and R. BALLENTINE, *Rev. Sci. Instr.* **21**, 158 (1950).
8. S. C. BROWN and W. W. MILLER, *Rev. Sci. Instr.* **18**, 496 (1947).
9. M. L. EIDINOFF, *Anal. Chem.* **22**, 529 (1950).
10. D. F. WHITE, I. G. CAMPBELL, and P. R. PAYNE, *Nature* **166**, 628 (1950).
11. D. D. VAN SLYKE, R. STEELE, and J. PLAZIN, *J. Biol. Chem.* **192**, 769 (1951).
12. A. J. FREEDMAN and E. C. ANDERSON, *Nucleonics* **10**, No. 8, 57 (1952).

13. J. E. S. BRADLEY, R. C. HOLLOWAY, and A. S. MCFARLANE, *Biochem. J.* **57**, 192 (1954).
14. W. W. MILLER, R. BALLENTINE, W. BERNSTEIN, L. FRIEDMAN, A. O. NIER, and R. D. EVANS. *Phys. Rev.* **77**, 714 (1950).
15. R. C. HAWKINGS, R. F. HUNTER, and W. B. MANN, *Can. J. Research* **27B**, 555 (1949).
16. W. B. MANN and G. B. PARKINSON, *Rev. Sci. Instr.* **20**, 41 (1949).
17. W. F. LIBBY, E. C. ANDERSON, and J. R. ARNOLD, *Science* **109**, 227 (1949).
18. H. BARKER, *Nature* **172**, 631 (1953).
19. A. R. CRATHORN, *Nature* **172**, 632 (1953).
20. W. J. ARROL and R. F. GLASCOCK, *J. Chem. Soc.* **1948**, 1534.

## CHAPTER IV

### GAS PHASE ASSAY OF CARBON-14 (CONTINUED)

#### GEIGER COUNTING OF CARBON DIOXIDE

##### *Method of Brown and Miller*

The poor counting properties of carbon dioxide in the proportional region are even more marked at higher voltages, and no Geiger region is observed. The behavior of counters filled with carbon dioxide alone is attributed, by Brown and Miller (1), to the formation of negative ions through the capture by positive ions of two electrons at the cathode, carbon dioxide being one of the relatively few gases in which there is a reasonable probability of this occurring. Thus, as the positive ion sheath approaches the cylinder,  $\text{CO}_2^+$  ions are transformed into  $\text{CO}_2^-$  ions which are immediately repelled back towards the anode. This type of phenomenon is highly deleterious to counter action and is indicated by the production of spurious pulses. Since it can be shown by calculation that no conducting surface has a high enough work function to eliminate negative-ion formation in carbon dioxide, the only way of overcoming the difficulty is to devise a means of preventing the positive ions from reaching the cathode. It has been known for some time that ions of high ionization potential could be prevented from reaching the cathode by adding another gas of lower ionization potential. The positive charges on the molecules of the first gas are then transferred to molecules of this second gas, forming ions which are attracted to the cathode and neutralized without the formation of negative ions. Brown and Miller call this second gas the *charge-transfer gas*.

The introduction of a successful charge-transfer gas into a carbon dioxide filled counter should thus eliminate the spurious



counts arising from negative-ion formation and introduce a plateau into the characteristic. The more efficient the charge-transfer mechanism, the flatter the plateau. The figures in Table 3 are taken from Brown and Miller's paper and show that, with carbon disulphide, successful results were obtained; the plateau slope was only about 3% per 100 volts, which is about the value obtained for a self-quenching argon-alcohol filled counter.

TABLE 3

<i>Charge-transfer gas</i>	<i>Plateau slope % per 100 volts</i>	<i>Ionization potential (volts)</i>
Pyridine	6.5	9.8
Acetone	5.0	10.1
Carbon disulphide	3.0	10.4
Ammonia	8.5	10.5
Water	7.0	13.0

In this table are shown five of the many vapors tried by Brown and Miller as charge-transfer gases. The ionization potential of carbon dioxide is 14.4 volts, and it will be seen that the substances tried all have ionization potentials lower than this, as is required for the charge-transfer mechanism to be effective.

The effect of varying the proportion of carbon disulphide in a carbon dioxide-carbon disulphide filled counter is shown in Figs. 25 and 26. In Fig. 25, the starting voltage is plotted against the percentage of carbon disulphide in admixture with carbon dioxide at a fixed pressure of 20 cm. mercury. The least sufficient amount of carbon disulphide to effect complete charge transfer appears to correspond with the minimum value of the starting voltage. At higher concentrations, the counter increasingly resembles carbon disulphide filled counters, which have good counting characteristics but give smaller pulses. Thus with increasing carbon disulphide concentration, the pulses for a given voltage get smaller and hence the starting voltage must be higher for them to be large enough for the electronic equipment to detect.

The effect is shown in another way in Fig. 26 where it will be seen that long flat plateaus are obtained when the gas mixture contains 5% or more of carbon disulphide, and also that the Geiger threshold occurs at higher voltages with increasing carbon disul-

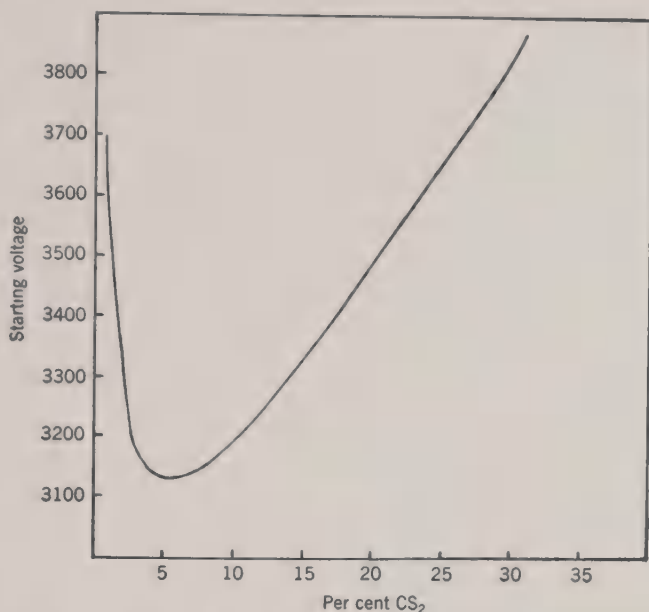


FIG. 25. Effect of carbon disulphide concentration on starting voltage (Brown and Miller). Starting voltage of a counter filled with CO<sub>2</sub> at 20 cm. pressure plotted against proportion of added CS<sub>2</sub>.

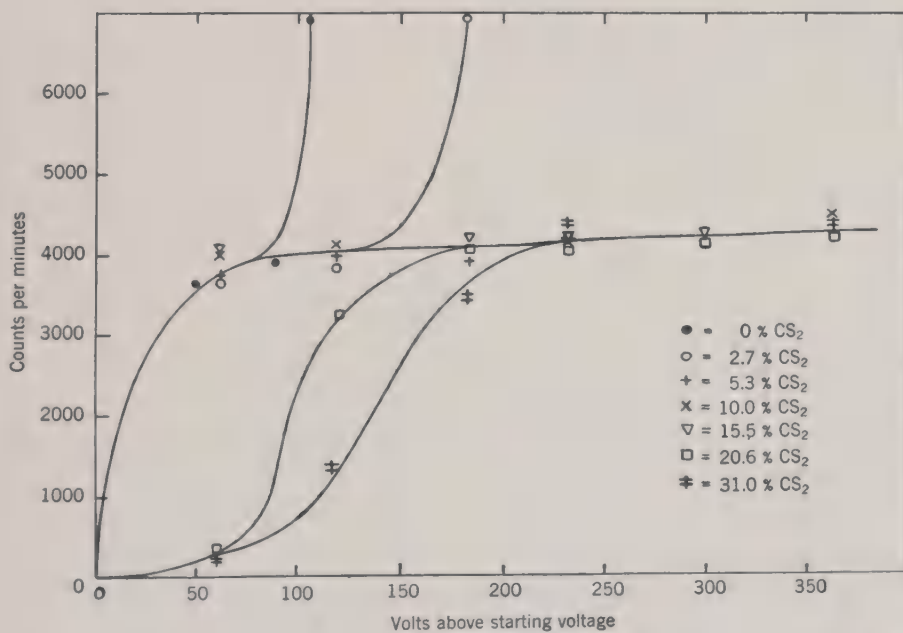


FIG. 26. Characteristics of CO<sub>2</sub>-CS<sub>2</sub> filled counters (Brown and Miller), showing effect of varying proportions of CS<sub>2</sub>.

phide concentration. It thus appears that 5% is about the minimum for the successful operation of these counters, and Brown and Miller recommend 10% for routine purposes, i.e., 20 cm. pressure of carbon dioxide plus 2 cm. pressure of carbon disulphide.

It should be noted that carbon disulphide does not function as a quenching agent; this is usually a substance, such as alcohol, with more than 3 atoms in its molecule, whose function is referred to on page 60. Carbon dioxide-carbon disulphide counters are not self-quenching and an external quench circuit is necessary for their operation. Brown and Miller recommend a Neher-Pickering (2) type of circuit, and the present author has found a Neher-Harper (3) circuit also satisfactory (see Fig. 20), so presumably either of these circuits may be used. For the successful counting of carbon dioxide in the Geiger region it is therefore necessary to install only the usual electronic equipment (high-voltage unit, scaler, and timer) together with a quenching circuit. No amplifier or discriminator is required. The counters are filled with carbon dioxide and carbon disulphide as described above. The present author uses this method in his laboratory for the routine determination of  $C^{14}$ , and the following description closely follows that of his actual apparatus and methods.

#### *Recommended Procedure for Brown and Miller Geiger Counting of Carbon Dioxide*

*Electronic equipment.* The conventional electronic equipment used for other types of Geiger counter is suitable for gas counting provided the high-voltage unit will give up to 3.5 kv., which is the operating voltage for counters filled at the convenient pressure of 22 cm. In addition to the scaler and high-voltage unit, a timer and an oscilloscope are desirable, the latter being permanently connected so as to monitor the input to the scaler. This is done because each time the counter is used it contains a new filling, and spurious counts due to interfering substances such as air can be more easily detected by studying the trace. The oscilloscope is also useful when putting a new counter into service since pulse size and operating voltage can then be correlated.

If the Neher-Harper quench circuit is used, it is important to

remember that the vacuum tube must be tested to 4 kv. In most commercial electronic equipment, provision is made on the scaler for supplying filament current and high voltage to such auxiliary devices as probes and quench units. The output from such devices and the filament current and high voltage are usually transmitted through wires carried in the same multi-core lead.

*Lead castle and connections.* If a lead castle suitable for housing a counter of the size and shape chosen cannot be obtained commercially then one can be constructed cheaply and conveniently along the lines suggested in Fig. 27. The annular space between two concentric brass tubes is filled with lead, the inner tube being of a diameter sufficient to accommodate all the counters in use, and the length being sufficient to shield the whole counter, leaving only the stopcock protruding. The "castle" contains at least an inch thickness of lead and is supported on a steel tripod. A pair of screw terminals supported on a Perspex panel is connected to a pair of concentric sockets carried on panels attached to the legs of the tripod. The leads from the quench circuit are plugged into these sockets. Thus the counter may readily and quickly be connected and disconnected from the electronic equipment. It has been found that not all plastics have a sufficient resistance to prevent leakage between the terminals; this leakage will then show up as a saw-tooth trace on the oscilloscope when the high voltage is applied. Perspex, however, is satisfactory provided the terminals are about 4 cm. apart. It should also be noted that as much as possible of all leads should be screened to minimize pickup.

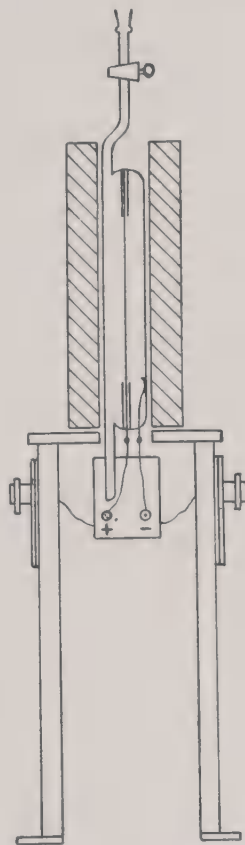


FIG. 27. Lead castle for gas counters, showing connections.



By taking this precaution, interference can be avoided, even when a high-frequency coil is being used in the same laboratory.

*Counters.* As stated in Chapter III, counters of about 2.5 cm. internal diameter, 15–16 cm. long, and with a total length of about 35 cm. are recommended (see Fig. 22). Both the anode and cathode tungsten rods should protrude far enough to allow flexible leads to be hard soldered to them without damaging the glass. Much trouble occurs if the makers fail to observe this point. Flexible leads often break off with continued use, and it is very difficult to attach new ones if insufficient electrode is available.

The graphite cathode film should extend beyond the points of emergence of the anode wire from its protecting sleeves to avoid erratic performance due to end effects. The graphite should have been well polished during manufacture to eliminate points from which discharges might take place.

The tube into which the counter filling is condensed is necessarily eccentric and should be about 4 cm. long and 6–7 mm. in internal diameter. The point of attachment of the stopcock must also be eccentric, but if the glass tubing is bent so that the mid-point of the stopcock is co-axial with the counter, a more compact instrument of better appearance results and is one which, furthermore, is less easily broken than one of a less symmetrical design.

#### *Putting New Counters into Service*

The counter is usually received without its stopcock and is either open to the air or contains the argon-alcohol filling by means of which the makers have tested it. The demand for gas counters is at present so small that manufacturers do not find it worth while to install apparatus for charging counters with other than the conventional filling. Although this is, of course, better than not testing them at all, the results are not necessarily a reliable guide to the performance of the counter with a different filling. Some manufacturers, however, will agree to testing the counter with their argon filling at a sufficiently high pressure to necessitate the same voltage as the customer intends to use (3–4 kv.). This will detect those whose electrical properties are poor.

The new counter is opened and the stopcock and standard

joint attached to one end and the condensation tube to the other. It is then baked out in high vacuum for at least an hour at  $300^{\circ}$ . A mere warming with the hand torch is usually insufficient and may be positively dangerous since cracking of the tungsten-in-glass seals can easily occur under too sudden local heating. It is best to provide a special vacuum manifold as shown in Fig. 28 to which counters can be attached with stopcock undermost while being baked out in a tubular heater. This preliminary heating is

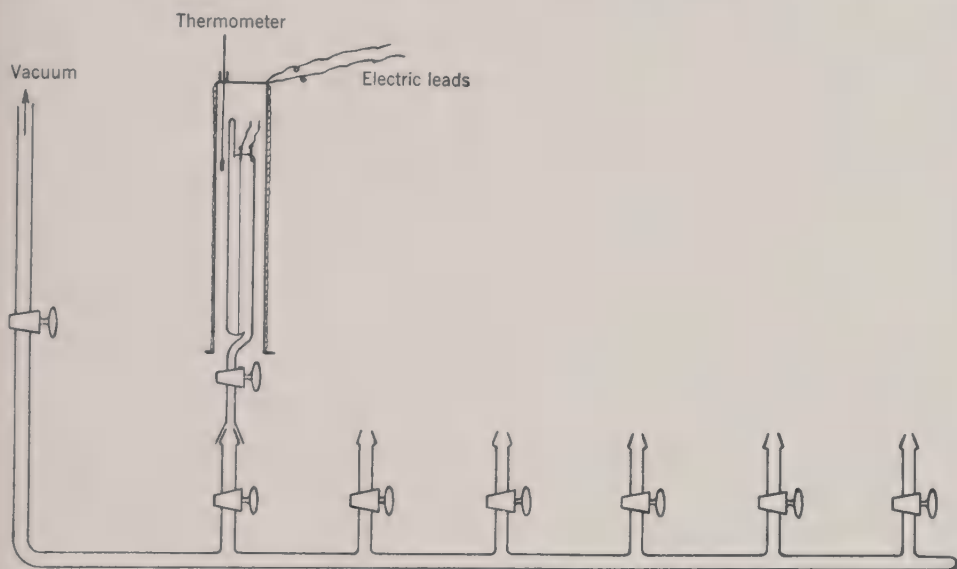


FIG. 28. Baking-out manifold for gas counters.

absolutely essential for graphite-cathode counters and presumably gets rid of oxygen which, chemisorbed on the cathode, causes spurious counts by negative-ion formation. With metal-foil cathode counters, baking is usually, although not always, necessary, and in this case promotes the outgassing of the metal.

The stopcock may be greased with either silicone or standard high-vacuum grease but, if the latter is used, protection from heat during baking is necessary, especially if the counter is not supported with the stopcock downwards.

It is sometimes found that a counter which has been working perfectly since it was first put into service perhaps a week earlier will paralyze—that is to say, it will stop delivering pulses. It will

often be found that the voltage has fallen below the operating value for the counter and cannot be raised because the counter is apparently passing more current than the power unit can deliver at the higher voltage. Warming and drying the glass around the electrodes brings about a temporary recovery and indicates that the cause of the trouble is a film of water. This may be prevented and a permanent cure effected by treating the glass around the electrodes with a suitable water-repellent silicone preparation such as Dow Corning silicone fluid DC 1107. In the author's laboratory the electrode end of every new counter is dipped into a 5% solution of this material in carbon tetrachloride before baking, thus outgassing the cathode and fixing the film at the same time.

The counter, having been given this preliminary treatment, is filled with carbon disulphide (2 cm. mercury pressure) and carbon dioxide (20 cm.) in the manner described below and connected up in the lead castle, taking care to connect the center wire to the positive terminal. Permanent damage may be done to a counter when the high voltage is switched on if the electrodes are connected the wrong way round. A good counter will begin to give pulses large enough to be recorded by the scaler at about 2800 volts and, as the voltage is increased up to about 4 kv., will give bigger but not notably more numerous pulses. Over the range 3–4 kv. there should be the plateau background counting rate of about 50 counts/min. A counter is sometimes obtained which does not behave in this way but gives increasing numbers of pulses with rising voltage until, towards 4000 volts, they are produced in vast numbers. Sometimes the trouble is irremediable and the counter must be rejected; sometimes further baking *in vacuo* will cure it; sometimes it is found to be due to an undiscovered leak that permits the entry of air. The discovery of some incondensable gas in a counter which is not behaving properly should always lead to a search for leaks.

If a counter stands up to this preliminary test, its characteristic should be plotted, using either an external source or, better, a sample of standard active gas. The use of a sample of standard active gas has the advantage that at the same time as the charac-



teristic is being determined the efficiency of the counter with respect to a standard counter can be determined also. Not all counters have exactly the same geometry and efficiency, and a standard filling will give different counting rates in different counters. Thus it is useful to put about six counters into commission at a time, keeping one of them as a standard against which the efficiency of all the others and all future counters can be recorded. Then the counting rate which a particular filling in any counter would have if transferred to the standard counter can be quickly found by using the appropriate factor. A filling that counts at 1000 per minute in the standard counter may give a counting rate of as little as 900 or as much as 1100 per minute in any other counter. In all experimental work, the activity of a given sample should be recorded with reference to the standard counter, thus making all experimental values comparable. A convenient source of radioactive carbon dioxide is an organic substance such as glucose with a specific activity of about 1000 counts/min./mg. carbon. A 10- to 20-mg. sample can then be combusted whenever a sample of standard radioactive carbon dioxide is needed.

The characteristic of every new counter should be plotted and the record kept on file. It should show a well-defined plateau extending over at least 400 volts and having a slope of not more than 3% per 100 volts. The operating voltage should be fixed about half-way along the plateau.

#### *Counter-filling Apparatus on Permanent High-vacuum Apparatus*

The filling apparatus is shown in Fig. 29. The space bounded by stopcocks  $S_{16}$ ,  $S_{17}$ ,  $S_{18}$ , the manometer, and the mercury cut-off is calibrated, pressure against volume, as described in Chapter II. In order to show the layout of this part of the apparatus on paper, the tube connecting the bulb of carbon dioxide, which is behind the plane of the other apparatus, is shown connected to it from the left. In practice it is best to have it connected from behind, thus running in a line perpendicular to the plane of the paper. The manometer is also behind the plane of the apparatus in a



position where it is least likely to get accidentally knocked. Whatever manner of making these connections is chosen, however, the aim should be to combine convenience of operation with making the volume of the dead space  $X_2$  no more than 30 ml.

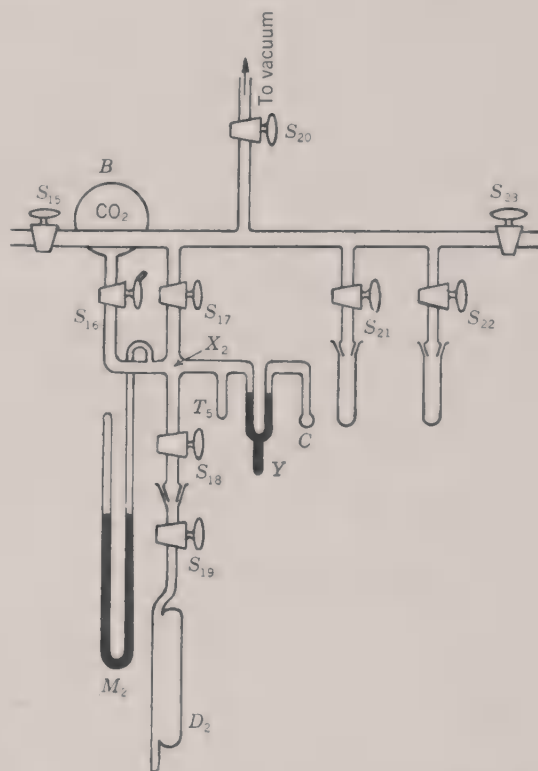


FIG. 29. Gas-counter filling manifold for the filling of  $\text{CO}_2$  counters (part of the main apparatus shown in Fig. 1).  $B$ :  $\text{CO}_2$  reservoir;  $C$ :  $\text{CS}_2$  reservoir;  $D_2$ : gas counter;  $M_2$ : manometer;  $S_{15}$ – $S_{23}$ : stopcocks;  $T_5$ : condensing trap for manometric chamber;  $X_2$ : manometric chamber (calibrated space bounded by  $S_{16}$ ,  $S_{17}$ , and  $S_{18}$ ,  $M_2$ , and  $Y$ );  $Y$ : mercury cut-off.

Then a reading of about 2.5 cm. on the manometer corresponds with about 1 standard ml. of gas.

*Carbon disulphide reservoir.* After the apparatus has been built and tested for leaks it is pumped out hard with the mercury cut-off open. Carbon disulphide, previously dried and redistilled,

is then charged into the bulb *C* by a vacuum distillation from a tube attached to the joint below stopcock *S*<sub>18</sub>. This is a better method of introducing the carbon disulphide into *C* than by pouring it in through a side arm and then sealing off in the blow-pipe; even with the carbon disulphide frozen down with liquid air as a precaution against fire, uncondensed vapor in the air is pyrolyzed and causes an unpleasant mess on the glass. Distillation *in vacuo* carries over a certain amount of mercury vapor which appears as a black film in the bulb but which coalesces to an insignificant drop on warming up.

*Carbon dioxide reservoir.* This consists of a 1- or 2-liter bulb connected to *X*<sub>2</sub> through a control stopcock of the pattern already described in Chapter II. Commercial solid carbon dioxide is a convenient source of the gas. It is allowed to evaporate into the evacuated bulb from a tube attached to the standard joint below stopcock *S*<sub>18</sub>. Solid carbon dioxide always contains some frozen water and, consequently, an excess should be used to ensure that the water remains frozen right to the last. The process of evaporation and filling of the bulb can be watched on the manometer and usually takes an hour or so. When the bulb is full, a sample should be withdrawn and frozen down in the trap *T*<sub>6</sub> to make sure it is free from air. If any of the sample remains uncondensed, then the whole batch should be frozen down again, the air pumped off, and the gas allowed to evaporate once more into the bulb.

*Filling the counter.* The carbon disulphide bulb *C* is chilled in liquid air and the mercury cut-off is opened. The carbon disulphide is then allowed to warm up with the stopcocks *S*<sub>18</sub> and *S*<sub>19</sub> open, but with *S*<sub>16</sub> and *S*<sub>17</sub> closed. When the manometer shows 2 cm. pressure, the counter stopcock, *S*<sub>19</sub>, is closed and the vapor in the dead space *X*<sub>2</sub> condensed back into *C* and the mercury cut-off closed.

Carbon dioxide from *B* is then cautiously admitted into *X*<sub>2</sub> through *S*<sub>16</sub> so that the manometer mercury rises slowly. When it is at about the 10-cm. mark, the counter stopcock *S*<sub>19</sub> is opened, admitting gas to the counter. The pressure is then quickly adjusted to 22 cm. and the counter stopcock *S*<sub>19</sub> closed. It is neces-

sary to wait until the carbon dioxide pressure is considerably greater than the carbon disulphide pressure in the counter before opening the counter stopcock, otherwise some carbon disulphide vapor will flow back into  $X_2$  and be left behind when the counter is removed.

This is the easiest way of filling a counter when a background is to be determined, as it avoids the processes of chilling the condensing tube and then drying the lower end of the counter. When a radioactive sample is to be included in the filling, however, it is convenient to know what pressure of gas must be in the counter plus manometric space  $X_2$  to give 22 cm. pressure when all is transferred to the counter. This is easily found out by pumping off the excess gas after filling a counter as described above and then opening the counter stopcock again and allowing the filling to expand into  $X_2$ . The pressure is noted, and, as it is about the same for all counters of similar size, it is convenient to mark the manometer scale. When a radioactive sample is to be included in the filling, the counter is first charged with carbon disulphide as already described and then, with the stopcock  $S_{18}$  closed, the radioactive sample is measured in the manometric space  $X_2$ . The stopcocks  $S_{18}$  and  $S_{19}$  are then opened, the pressure adjusted to the mark with inactive carbon dioxide, and the whole gas mixture condensed into the counter. Stopcocks  $S_{18}$  and  $S_{19}$  are then closed, the counter detached from the apparatus, and, after warming up and drying, connected up in the lead castle.

This filling procedure may be used for all radioactive samples, but if the sample is less than about 1 ml., and therefore measured in the McLeod gauge, it may be introduced into a counter which already contains a background filling. A few per cent increase in pressure will not appreciably move the operating voltage along the plateau and will cause no measurable error.

#### REFERENCES

1. S. C. BROWN and W. W. MILLER, *Rev. Sci. Instr.* **18**, 496 (1947).
2. H. V. NEHER and W. H. PICKERING, *Phys. Rev.* **53**, 316 (1938).
3. H. V. NEHER and W. W. HARPER, *Phys. Rev.* **49**, 940 (1936).

## CHAPTER V

### COMBUSTION OF LABELED COMPOUNDS

For the assay of the isotopes of carbon and hydrogen by gas analysis, the sample must first be combusted. All that is really required is a representative sample of the carbon and hydrogen in the form of carbon dioxide and water but, as it has been shown that isotopic fractionation can occur during the course of both wet and dry combustions, it is obviously best if the production and collection of products is quantitative. Armstrong and co-workers (1) found that carbon dioxide fractions collected at different times during the course of the combustion of xanthidol- $C^{14}$ -ureide had different specific activities. They attributed this result to some parts of the molecule being more readily combustible than others or (in wet combustion) to an initial splitting of the molecule into labeled and unlabeled moieties and their combustion at different rates. It would therefore seem advisable to guard against such occurrences in the combustion of other compounds also.

Since the quantitative combustion of small quantities of organic compounds is regularly carried out in many laboratories for the purpose of microanalysis, there is a copious literature on the subject. Workers with isotopes requiring carbon dioxide and water samples for isotopic analysis have therefore usually found it convenient to adapt existing methods of combustion to their needs. In recent years many papers have appeared describing methods for the simultaneous determination of total carbon and hydrogen and the abundance of their isotopes. Many of these methods appear to differ only in insignificant details, and an attempt will be made here to give only representative examples.

Workers concerned only with the determination of carbon and its isotopes have a choice between the wet and dry methods



of combustion usually associated with the names of D. D. Van Slyke and F. Pregl, respectively.

The Van Slyke wet combustion method depends on oxidation with a combustion fluid based on chromic acid. Carbon dioxide, which is measured manometrically, is the only product which can be collected, the water being lost into the reagent. Van Slyke himself has introduced suitable modifications into his combustion and manometric methods to enable the activity of the carbon dioxide to be measured.

Pregl, who died in 1930 was, of course, concerned only with the quantitative determination of the elements in organic compounds, and his methods are based on the combustion of the sample in a stream of oxygen and the collection of the products in chemical traps. The modifications of his method introduced recently for the purpose of isotopic gas analysis have been chiefly in the means used for stripping the products of combustion from the oxygen stream; liquid-air chilled traps have replaced bulbs of calcium chloride and soda lime.

### Wet Combustion Methods

#### *Method of Van Slyke and Folch*

Van Slyke's well-known manometric apparatus, when applied to the determination of  $C^{14}$ , has the advantage over many others of being reasonably compact and therefore usable in an ordinary laboratory without special accommodation. Having been adapted to the determination of radioactive carbon from a method originally designed only for the manometric determination of total carbon, it is probably not as convenient as it would have been had it been designed for its dual purpose from the start. Thus, to the present author, it seems wasteful of time to produce carbon dioxide by combustion, to absorb it in alkali, and then to liberate it for measurement and counting. The separation of carbon dioxide from other gases and its quantitative transfer to a manometric chamber can be accomplished much more conveniently by the application of high-vacuum techniques. On the other hand, it is to be noted that the manometric apparatus can

be operated without pumps, which is a distinct advantage if only total-carbon determination is required or if solid counting is to be used. Pumps must, however, be used for gas counting, thus making the Van Slyke technique, as a whole, probably less convenient than others which will be considered.

The manometric apparatus was first described in 1924 (2) but has been much improved in detail since then. The most recent information is contained in three papers published in the *Journal of Biological Chemistry* and conveniently bound together as reprints by the authorities of the Brookhaven National Laboratory.

The recipe for the combustion fluid is modified from time to time by Van Slyke and his collaborators. One recipe is given verbatim below and is taken from the first of the three papers cited (3). It contains all the ingredients in a single reagent but has rather poor keeping properties. This difficulty has been overcome by a procedure described in the second of the three papers in which some of the ingredients are omitted from the liquid reagent and added in the solid state to the combustion tube.

*Combustion fluid.* "In a 1 litre Pyrex Erlenmeyer flash, provided with a ground glass stopper, place 25 g. of  $\text{CrO}_3$  and 5 g. of  $\text{KIO}_3$  followed by 167 cc. of syrupy phosphoric acid (Sp. gr. 1.7) and 333 cc. of *fuming* sulphuric acid (20 per cent free  $\text{SO}_3$ ). Leaving the stopper off, heat the mixture on a wire gauze until the temperature reaches  $140^\circ$ – $150^\circ$ . Rotate the flask gently at times to assist solution of the chromic anhydride and escape of  $\text{CO}_2$  formed by oxidation of any organic matter that may be present. When  $150^\circ$  has been reached remove the flame, cover the open flask with an inverted, lipless 150 cc. beaker and let the mixture cool to room temperature. When cool insert the glass stopper but also keep the inverted beaker permanently over the stopper to prevent dust from settling on the rim of the flask and contaminating the fluid as it is poured out."

*Apparatus and technique.* The Van Slyke manometric apparatus is shown in Fig. 30, and the additional apparatus required for filling the counter with carbon dioxide and adjusting the pressure with methane in Figs. 31 and 32.

The principle of the method is as follows: Oxidation takes

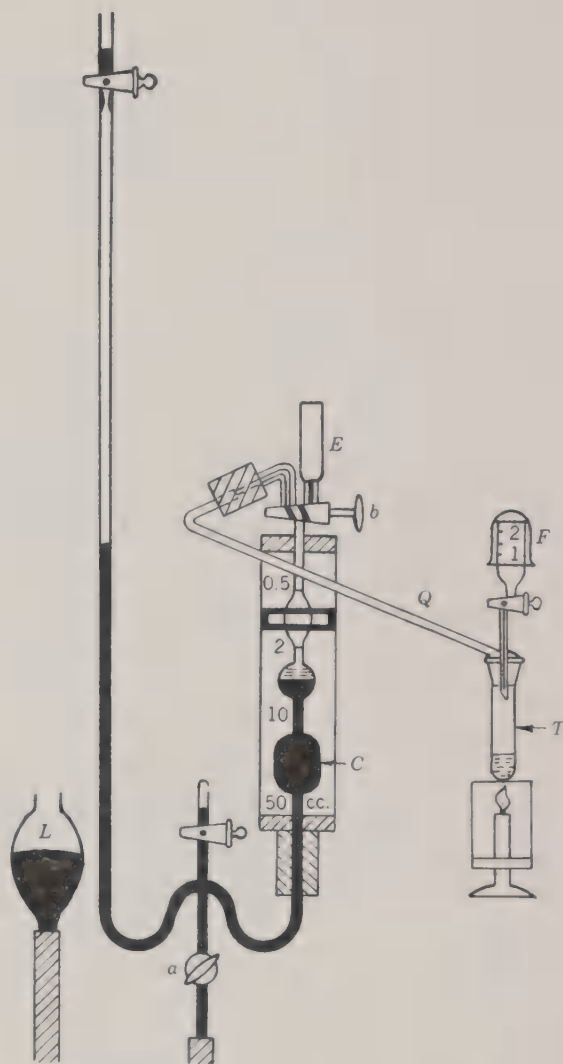


FIG. 30. Wet combustion apparatus (Van Slyke and Folch). *a*: stopcock on mercury supply; *b*: two-way stopcock on manometric chamber; *C*: manometric chamber marked at 0.5, 2, 10, and 50 cc.; *F*: dropping funnel for combustion fluid; *L*: mercury leveling bulb; *Q*: delivery tube; *T*: combustion vessel; *E*: cup for forming Hg seal.

place in the combustion tube *T*, and the resultant carbon dioxide is absorbed in alkaline hydrazine solution which is floated on the mercury in the manometric chamber, *C*. Hydrazine is used in order to reduce and retain any free halogens which might otherwise be formed and introduce an error into the measurements. The carbon dioxide is then re-liberated in the manometric chamber by adding acid, the pressure it exerts is measured, and its volume is calculated.

A weighed amount of sample containing carbon in the range 0.1–15 mg. is transferred to the combustion tube *T* and a few pieces of cleaned alundum added to promote smooth boiling. A standard taper joint, carrying a delivery tube *Q* and a tap funnel, is attached to the combustion tube, both stopcock and joint being lubricated with syrupy phosphoric acid. The delivery tube is connected to one arm of the two-way stopcock *b*. The combustion fluid is charged into the funnel. The amount used depends on the weight of carbon to be oxidized and is at the rate of about 1 ml. for every 3 mg. of carbon.

It is desirable to remove much of the air from the combustion chamber since this diminishes the blank due to atmospheric carbon dioxide and facilitates, later in the analysis, the transfer of carbon dioxide from the combustion tube to the alkaline solution in the manometric chamber. Since no pumps are used on the manometric apparatus proper, the air is removed by using the manometer mercury as a pump and the stopcock *b* as a valve. This stopcock is turned to connect the combustion tube with the manometric chamber and the mercury is lowered, thus withdrawing about two-thirds of the air. The stopcock is then readjusted so that this air is ejected when the mercury is raised. The procedure is repeated until about 90% of the air has been removed.

Alkaline hydrazine solution (2 ml. 0.5 *N* carbon dioxide-free sodium hydroxide + 0.3 *M* hydrazine) is then introduced into the manometric chamber. A tap funnel with a rubber tip on the end of the stem is held so that the rubber tip is pressed firmly against the bottom of the cup *E*. Mercury is poured around it, making a seal, and the stopcock *b* is opened wide. By controlling the flow of mercury through the stopcock *a* to the leveling bulb



*L*, the admission of alkali to the chamber may best be regulated. The cup *E* is rinsed with acidified water immediately after this procedure so that it does not stand wet with alkali.

*Combustion.* By lowering the leveling bulb and manipulating the stopcock *a*, the mercury in the manometer is lowered until it stands level with the 2-ml. mark on the manometric chamber. The bulb is clamped so that the surface of the mercury in it stands opposite the 50-ml. mark, and stopcock *a* is closed. This setting of the apparatus is most convenient for observing pressure changes occurring during the combustion. Stopcock *b* is then turned so that the manometric chamber communicates with the combustion tube. The measured volume of combustion fluid is run in and the tube gently heated with a micro-burner. The fluid boils in about 2 min., the rate of heating being controlled so that the foam collar due to gas evolution is never more than 2 cm. high.

As carbon dioxide and oxygen are evolved, the mercury level falls in the manometric chamber and rises in the manometer. It is adjusted from time to time by means of the leveling bulb so that the gas space in the manometric chamber is kept at about 1 ml. After about a minute, the pressure is such that the mercury stands at its full height in the manometer. The stopcock *a* may then be kept open without danger of the mercury and alkali in the manometric chamber flowing over into the combustion tube. Boiling at this pressure (about 60 cm.) is continued for another 1.5 min. by which time all the substances tested by Van Slyke and Folch were completely oxidized. Boiling beyond the specified time brings about the production of excessive amounts of iodine vapor and oxygen.

*Absorption, re-liberation, and measurement of carbon dioxide.* With the flame still under the combustion tube, the mercury in the manometric chamber is raised and lowered about 20 times between the 5- and 50-ml. marks to bring about the complete absorption of the carbon dioxide. The delivery tube is then disconnected from the two-way stopcock *b* and the unabsorbed gases ejected through it by raising the mercury. A volume of 2 *N* lactic acid exactly equivalent to the alkali is next introduced into the

manometric chamber using a mercury seal and a rubber-tipped delivery funnel exactly as described for the admission of the alkali. The mercury is lowered to the 50-ml. mark and the chamber shaken for about half a minute to permit evolution of most of the carbon dioxide. The mercury level, which has been depressed by the gas, is restored to the 50-ml. mark and shaking continued for a further 1.5 min. The solution is then raised exactly to the 50-, 10-, or 2-ml. mark, depending on the volume of gas, and the reading on the manometer taken. The pressure is corrected by subtracting the saturated water vapor pressure from this reading, and the volume of carbon dioxide is calculated by multiplying by a previously determined factor. In the accurate microdetermination of total carbon, the carbon dioxide is reabsorbed in alkali and a further correction made for unabsorbed gas as well as for the fraction that remains dissolved in the aqueous solution. For the determination of  $C^{14}$  activity, however, the next stage recommended by Van Slyke and his colleagues, if gas counting is to be used, is the introduction of the carbon dioxide into a Bernstein and Ballentine type proportional gas counter. (See page 66.) This operates on a methane-carbon dioxide mixture at atmospheric pressure.

*Filling the counter.* The transfer to a counter is achieved by means of the auxiliary apparatus shown in Fig. 31. The previously evacuated counter is attached by means of a standard taper joint to the transfer line which itself is evacuated through stopcock *J*. The lower two-thirds of the drying coil *D* is cooled in a solid carbon dioxide bath and the bottom of the counter chilled in liquid air. Intervening stopcocks are then opened and the carbon dioxide, dried on the way by the chilled coil, is condensed into the counter. To ensure complete transfer, these workers recommend raising and lowering the mercury in the manometric chamber several times, although this should not be necessary if no in-condensable gases are present. The counter is then removed and attached to joint *A* of the methane reservoir (Fig. 32) and evacuated through stopcock *B*. Methane is imprisoned above oil in the center well and hence, the tube *G* being open to the air, is driven into the counter when stopcocks *D* and *K* are opened. This

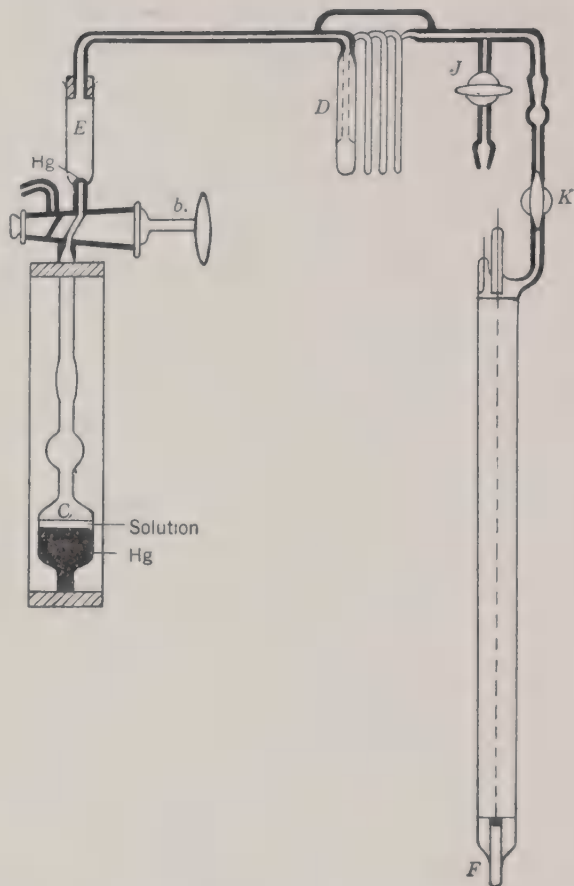


FIG. 31. Gas transfer line and counter (Van Slyke, Steele, and Plazin). *C*, *b*, and *E* are the manometric chamber, etc., as in Fig. 30 (the blob of mercury in *E* remains from the introduction, through a Hg seal, of acid for the reliberation of  $\text{CO}_2$  from the alkaline hydrazine solution). *D*: freezing coil for drying gas; *J*, *K*: stopcocks; *F*: Bernstein and Ballentine-type proportional counter.

is indicated by a rise in the oil level in the well *H*. The appropriate stopcocks are then closed, and the counter is removed and attached to the electronic equipment for counting. No provision is made for dividing the carbon dioxide into portions and counting them separately.

*Preparation of barium radiocarbonate.* If solid counting is preferred, the carbon dioxide in the manometric chamber may

be transferred to barium hydroxide solution instead of directly into a gas counter. In the present author's experience there is no advantage in doing so if gas counting apparatus is available since it involves the extra operations of filtration and preparation of the disk for solid counting. Since, however, the Van Slyke manometer

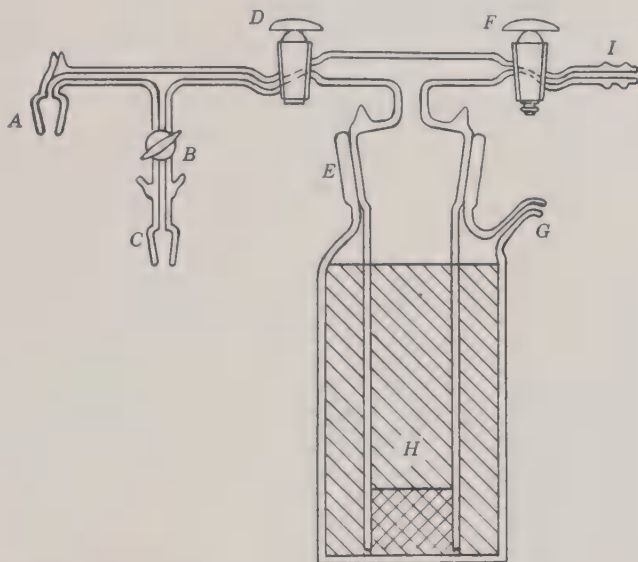


FIG. 32. Vessel for supplying dry methane at atmospheric pressure (Van Slyke, Steele, and Plazin). The cross-hatched space at the bottom of the center well *H* indicates the level of oil when the reservoir is charged with methane before filling a counter. The reservoir is made from a 2-liter bottle with standard joint. *A*, *C*: standard taper joints; *B*, *D*, *F*: stopcocks; *G*: Side arm open to atmosphere; *I*: tube for charging reservoir with methane.

metric apparatus can be used without pumps and high-vacuum line, solid counting may be preferred by some workers.

The additional apparatus designed by Van Slyke and co-workers is shown in Fig. 33. The tube *A*, of about 25 ml. capacity, contains 2.5 ml. of a barium hydroxide-barium chloride solution ( $0.25\text{ N} + 2\text{ gm. per } 100\text{ ml.}$ ) and is attached to a standard joint. This joint is connected to stopcock *G* which in turn may be connected to the cup *E* of the main manometric apparatus. In order



to transfer the radioactive carbon dioxide from the manometric chamber to the barium hydroxide solution, the pressure in *A* and *E* is first reduced by evacuating through the stopcock *H* with a filter pump. The two-way stopcock *b* on the manometric chamber is then opened and the mercury raised until the surface of the

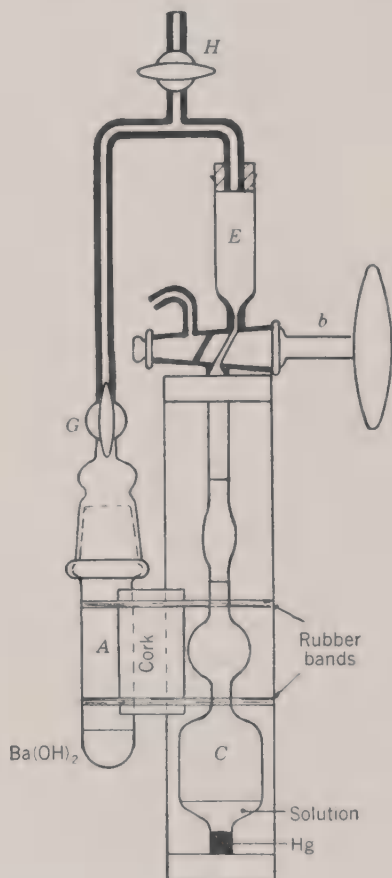


FIG. 33. Preparation of barium carbonate from carbon dioxide, showing manometric chamber, etc., as in Fig. 30, connected to tube of barium hydroxide solution (*A*).

aqueous solution floating on it reaches the bottom of the stopcock. The stopcock is then turned off and the mercury lowered and the apparatus shaken for 1.5 min. This promotes the absorption of the carbon dioxide in the barium hydroxide and, at the same time, the liberation of most of the carbon dioxide (about 5%) which remains dissolved in the aqueous solution in the manometric chamber. This gas is then transferred to the absorption tube and the whole procedure repeated to complete the transfer.

Finally, the tube *A* is removed and the contents immediately neutralized with *N*/10 hydrochloric acid to phenolphthalein indicator. The precipitate of barium carbonate is then filtered off and counted immediately or stored with the tube tightly stoppered until required.

*Wet Combustion-Solid Counting Method of Lindenbaum, Schubert, and Armstrong*

By using gravimetric determination of carbon instead of the manometric method, Lindenbaum and co-workers (4) have achieved a notable simplification of the wet combustion method since the barium carbonate produced can be used directly for solid counting. Their apparatus consists only of the items shown in Fig. 34. The sample of organic material containing not more than 15 mg. carbon is introduced into the 30-ml. combustion tube *E*. A tap funnel *B*, containing 10 ml. of combustion fluid and carrying a delivery tube beneath its stopcock, is attached to the combustion tube by means of a standard joint *D*, both joint and stopcock being lubricated with syrupy phosphoric acid. The delivery tube is connected, also by means of a standard joint, with the receiver *H* (a 100-ml. centrifuge tube) which contains 25 ml. of saturated barium hydroxide solution. This solution has been previously filtered into the tube by means of the filter assembly *K* shown above it in the figure. The barium hydroxide solution is cooled in melting ice and the combustion fluid run on to the sample. The apparatus is evacuated with a filter pump through stopcock *L* which is then closed and the contents of the combustion tube heated and kept boiling for 3 min. The apparatus is allowed to stand for 20 min., to ensure the complete absorption of the carbon dioxide, and air is admitted through stopcock *C*. The tube *F* is rinsed into the receiver and the precipitate of barium carbonate centrifuged down. After washing, it is filtered off in a suitable gravimetric apparatus, weighed, and its radioactivity determined by solid counting. The recovery of barium carbonate in the examples cited by the authors of the paper was usually within 0.5% of theoretical, and the average deviation between duplicate determinations of radioactivity was 1.9%.

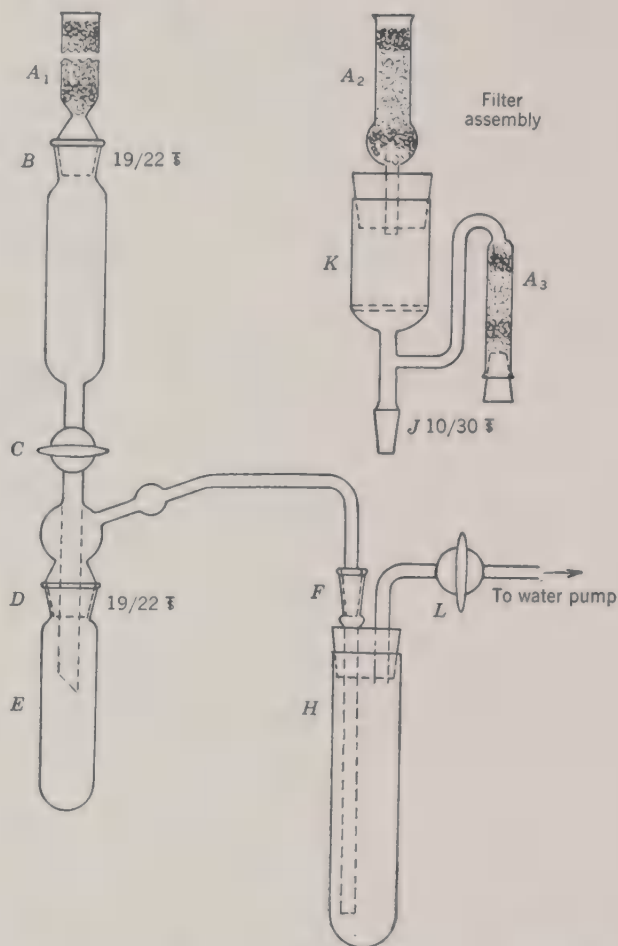


FIG. 34. Wet combustion apparatus (Lindenbaum, Schubert, and Armstrong).  $A_1$ ,  $A_2$ , and  $A_3$ :  $\text{CaCl}_2$  tubes;  $B$ : dropping funnel containing combustion fluid;  $E$ : combustion tube (30 ml.);  $H$ : Receiver (100-ml. centrifuge tube);  $C$ ,  $L$ : stopcocks;  $D$ ,  $F$ , and  $J$ : standard taper joints;  $K$ : sintered glass filter funnel. Barium hydroxide filtered into  $H$  by attaching joint  $J$  to socket  $F$ . Delivery tube on combustion apparatus is plugged into  $F$  and wet combustion then proceeds.

### *Wet Combustion Using Permanent Vacuum-line Technique*

Several workers have described wet combustion using a vacuum line to separate and measure the carbon dioxide produced. The method currently used in the present author's laboratory may be taken as an example. That part of the general pur-

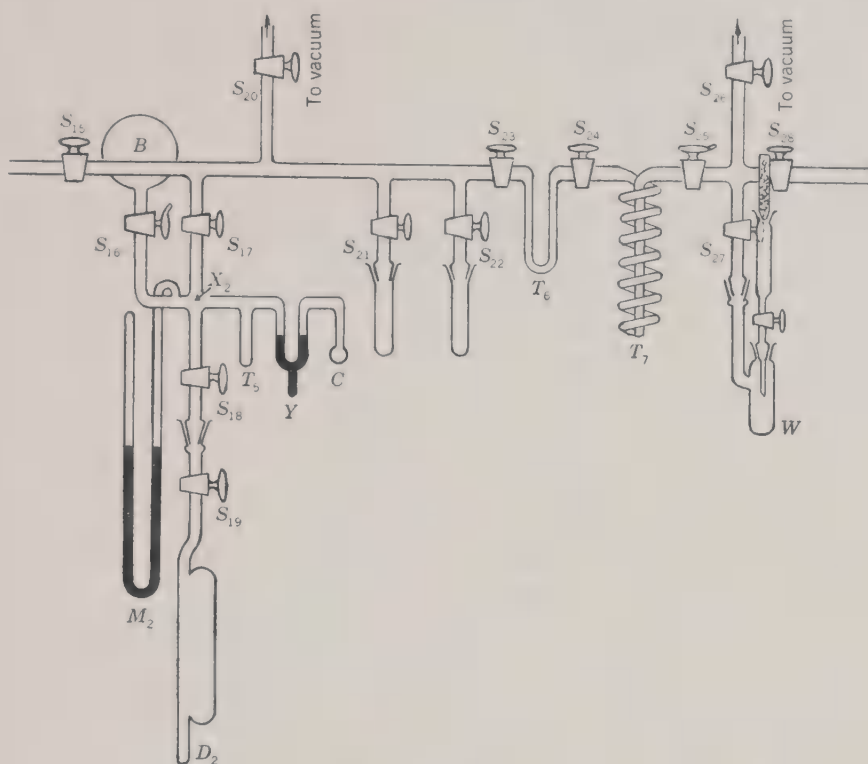


FIG. 35. Vacuum line and wet combustion apparatus, for wet combustion, gas measurement, and counter filling (part of main apparatus, shown in Fig. 1). The sample is wet combusted in *W* and the  $\text{CO}_2$  separated from incombustible gases by pumping through the traps, one or both of which are chilled in liquid air.  $\text{CO}_2$  is measured in *X*<sub>2</sub> and charged into the counter.

*B*:  $\text{CO}_2$  reservoir.

*C*:  $\text{CS}_2$  reservoir.

*D*<sub>2</sub>: Gas counter.

*M*<sub>2</sub>: Manometer.

*S*<sub>15</sub>–*S*<sub>28</sub>: Stopcocks.

*T*<sub>5</sub>: Condensation tube.

*T*<sub>6</sub>–*T*<sub>7</sub>: Traps.

*W*: Wet combustion apparatus (see Fig. 36).

*X*<sub>2</sub>: Manometric chamber.

*Y*: Mercury cut-off.

pose, high-vacuum apparatus (already described and illustrated in Fig. 1) which lies between *S*<sub>15</sub> and *S*<sub>28</sub> is used. This section of the apparatus, together with the wet combustion tube, which is attached to stopcock *S*<sub>27</sub>, is shown in Fig. 35.

The combustion tube itself, shown in greater detail in Fig. 36, has a capacity of about 50 ml. and is provided with a side arm bearing a standard taper joint. A tap funnel can be fitted to it by



means of another standard joint, the apparatus being constructed so that the end of the funnel reaches below the point of attachment of the side arm. The dimensions are such that the funnel stands clear of the working line when the device is attached to the stopcock  $S_{27}$ .

The sample to be combusted, which usually contains about 6

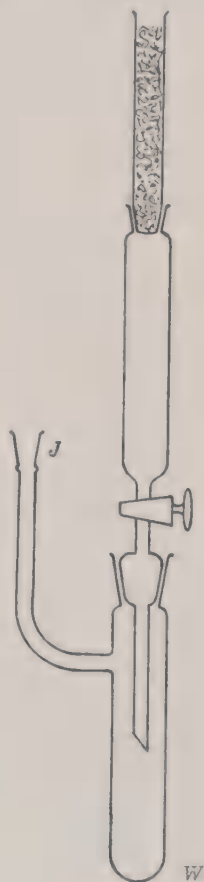


FIG. 36. Wet combustion apparatus, used with permanent vacuum line as shown in Fig. 35 by attaching the socket  $J$  to the cone below stopcock  $S_{27}$ .

mg. carbon, is weighed into the combustion tube and Van Slyke combustion fluid at the rate of 1 ml. for every 3 mg. carbon is introduced into the funnel, both joint  $J$  and the stopcock on the funnel being lubricated with syrupy phosphoric acid. The Van Slyke reagent is protected from atmospheric moisture by means of a calcium chloride tube. After attaching to the vacuum apparatus below the stopcock  $S_{27}$ , the combustion tube is evacuated. A high vacuum is not needed at this stage but evacuation must

be carried out carefully at first if the sample is light and likely to contain adsorbed gas, since rapid evacuation may scatter it about inside the apparatus. Stopcocks  $S_{26}$  and  $S_{27}$  are then closed, the combustion fluid is run in, heated, and maintained at its boiling point for 3 min. The U-trap  $T_6$  is chilled in liquid air and, with stopcocks  $S_{27}$ ,  $S_{24}$ , and  $S_{20}$  (to vacuum) open, the gas in the combustion tube pumped slowly, during the course of about 5 min., through the chilled trap, the rate of flow being controlled by means of stopcock  $S_{25}$ . Quantitative combustion and stripping will usually be achieved since the amount of incondensable gas present, chiefly oxygen, is small. However, as an extra precaution, the spiral trap  $T_7$  may also be chilled if desired. The U-trap is finally pumped off under high vacuum while still surrounded by liquid air, the appropriate stopcocks adjusted, and the carbon dioxide, which is already dry, transferred to the manometric chamber for measurement.

### Methods Using Dry Combustion

The dry combustion technique of F. Pregl was designed for the quantitative combustion of organic materials on the 3- to 5-mg. scale although smaller quantities could be handled if necessary. His methods have been considerably modified by later workers, especially those wishing to obtain the products of combustion for isotopic assay, and have in some instances been scaled up. Pregl's methods are inherently simple but require rigorous attention to many small details if the degree of accuracy ( $\pm 0.2\%$ ) which Pregl specified is to be attained.

In Pregl's apparatus for the determination of carbon and hydrogen only, carefully purified oxygen is passed at a controlled speed over the heated sample in a hard-glass combustion tube. Pregl and his pupils disliked quartz tubes because they become opaque too quickly. The tube has an outside diameter of 9.5 to 10.5 mm. and a length of 50 cm. It is packed with Pregl's "universal filling" by means of which Pregl and his collaborators could obtain the quantitative combustion of any organic substance. Other workers, however, have not always found this to be so (see later). The filling consists of the following ingredients, named in order from the absorption end of the tube: a layer of purified

lead dioxide (2 to 2.5 cm.), a layer of silver wire or wool (4 cm.), a layer of copper oxide-lead chromate mixture (14 cm.), and, finally, another layer of silver wire or wool (1.5 to 2 cm.) to keep the filling in place. The lead dioxide is provided with a separate, constant-temperature heating mortar because, according to Roth (5), it obstinately retains a certain amount of water which otherwise gives rise to erratic hydrogen values. The main filling is maintained at a temperature of  $550^{\circ} \pm 20^{\circ}$  by gas or electricity. The absorption apparatus consists of calcium chloride and soda-lime tubes of specified size and design. The rest of the apparatus, which must be carefully made and erected if accurate results are to be obtained, consists of devices for purifying the oxygen and controlling its rate of flow.

Three to five milligrams of sample, carefully weighed on the microbalance into a boat made of platinum, porcelain, or glass, are introduced into the combustion tube some 4 to 5 cm. distant from the layer of silver wool. The oxygen stream is passed through the train, and the sample is gently heated. Combustion is completed in the tube, where the silver wire retains halogens and the lead dioxide retains the oxides of nitrogen if any of these elements are present. The water and carbon dioxide are trapped in the previously weighed absorption tubes.

It is interesting to note that whereas Pregl and his colleagues consider lead dioxide to be an "absolutely reliable absorbing agent for the higher oxides of nitrogen" (ref. 5, p. 15) other workers, e.g., Naughton and Frodyma (6), find it to be "totally inadequate for the removal of nitrogen oxides" and use a separate unit in which they are reduced by means of heated, freshly reduced copper gauze.

#### THE PRODUCTION OF FREE CARBON DIOXIDE AND WATER BY COMBUSTION

##### *Method of Naughton and Frodyma*

This method (6) was not designed for use in isotopic assay but is the result of a successful attempt to develop an apparatus for the determination of carbon and hydrogen in ordinary micro-samples using "manometric and vacuum techniques with as little

modification of the familiar and well tried Pregl method as possible." The apparatus is shown in Fig. 37.

*Combustion tube.* This consists of a Pyrex glass combustion tube 60 cm. long and 1 cm. in diameter provided with a standard taper cone at one end. About half its length is packed with a filling devised by Niederl and Niederl (7) consisting of a roll of platinum wire, copper oxide, and electrodeposited silver crystals. The tube is inclined at an angle of  $30^\circ$  with the horizontal and sealed to the stripping train. When in use it is heated electrically to a temperature of  $650^\circ$ – $700^\circ$  and must, therefore, be made of a glass which will not collapse under vacuum at this temperature.

*Nitrogen oxide reducer.* The nitrogen oxide reduction system consists of a glass tube 8 cm. long and 1 cm. in diameter containing a roll of copper gauze 4 cm. long freshly reduced with methanol vapor at  $650^\circ$ , together with a 2-cm. section of copper oxide. This section can be isolated and by-passed by means of stopcocks as shown in Fig. 37 and is used only if the sample contains nitrogen.

*Oxygen supply.* The oxygen is obtained from a cylinder, and the purification train consists of a tube packed with Ascarite-Anhydrone, a tube containing heated copper oxide, another Ascarite-Anhydrone tube, and a liquid air-chilled trap.

*Traps.* The products of combustion are stripped from the excess of oxygen by cooling trap *o* with solid carbon dioxide and trap *p* with liquid nitrogen. It is not quite clear from the published account at what pressure this stripping occurs; Naughton and Frodyma state that the stopcocks are adjusted so that a pressure of 30 mm. is recorded on the manometer but they do not say whether they mean 30 mm. of mercury or a reading of 30 mm. which, on their two-liquid manometer would correspond with a pressure of about 2 mm. mercury. As they achieve quantitative stripping using only one trap at  $-195^\circ$ , the latter alternative seems the most probable since, in the present author's experience, even two liquid-air cooled traps will not quantitatively remove carbon dioxide from an oxygen stream at pressures above 2 mm. mercury.

*Manometric system.* Both water and carbon dioxide are measured manometrically using calibrated manometric chambers



$r + p$  and  $q + o$  connected in turn to the two-liquid manometer already mentioned (page 34). Care must of course be taken that the amount of water measured is insufficient to exert a pressure close to its saturated vapor pressure, otherwise deviations from the gas laws would introduce serious errors. The manometric

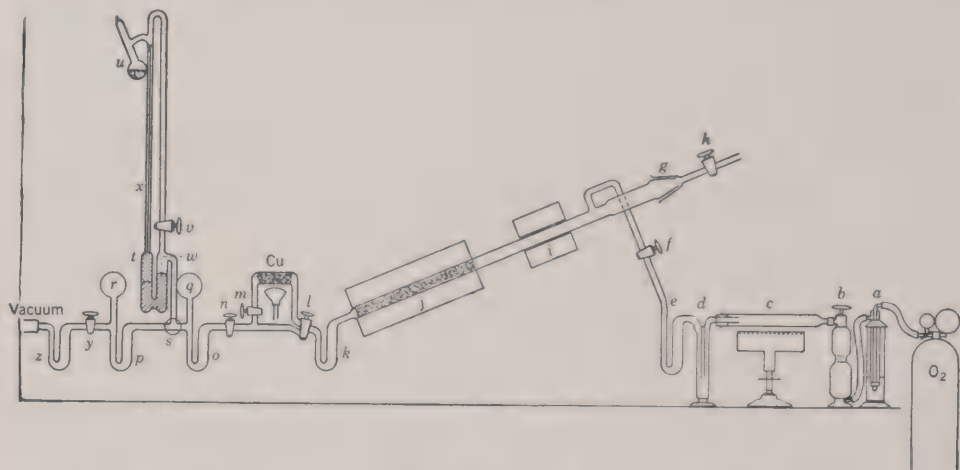


FIG. 37. Dry combustion apparatus (Naughton and Frodyma).

- |  |   |
|--|---|
| a: Oxygen pressure regulator.  | k, o, p, and z: U-traps.  |
| b: Oxygen purifier (Ascarite-Anhydron).                                | q and r: Calibrated volumes.  |
| c: Copper oxide-filled tube maintained at 500°.                        | t: Two-liquid manometer.  |
| Cu: Tube containing heated copper for reduction of oxides of nitrogen. | u: Oil storage flask.   |
| d: Ascarite-Anhydron tower.  | w: Mercury meniscus.  |
| e: Liquid air-chilled trap.  | x: Graduated limb of manometer.   |
| f, h, l, m, n, s, y, and v: Stopcocks.                                 | Manometer is pre-filled with clean mercury and the required amount of oil distilled in from u under vacuum. |
| g: Taper joint.  | q + o = manometric chamber for water vapor measurement.   |
| i: Sample heater.  | r + p = manometric chamber for carbon dioxide measurement.  |
| j: Combustion furnace.   |   |

chambers are calibrated empirically by running a series of combustions on high-purity benzoic acid (U.S. National Bureau of Standards).

*Procedure.* After "conditioning" the apparatus by running oxygen through it and ascertaining that zero blanks are obtained

an actual combustion may be carried out. One to five milligrams of sample is weighed into a boat and introduced into the combustion tube through the standard taper joint at *g*, afterwards replacing the cap bearing the stopcock *h*. Atmospheric gases which enter during this procedure are then swept out through *h* with oxygen and *h* is closed. Then, by regulating the oxygen supply and adjusting the stopcock *n*, the gas flow is controlled so that the pressure registered by the two-liquid manometer (now acting as a flow meter) is 30 mm. This corresponded, in Naughton and Frodyma's apparatus, to a flow of 5 ml./min. The sample is gently heated with a burner for 5 min. and stopcock *f* is closed. The system is evacuated through stopcock *n*, opening it gradually so that the gas flow through the stripping train does not exceed 5 ml./min. Then, by operating stopcock *f* again, gas is swept through the system at the same rate for a further 2 min. after which *f* is finally closed and the system evacuated as completely as possible. The carbon dioxide trap *p* is then isolated from the water trap *o* by means of stopcock *s*, and the pressures exerted by the two products in their respective manometric chambers are measured. By means of the calibration factors, these pressures can be converted into weights of water and carbon dioxide, and the composition of the sample can be calculated.

*Procedure with nitrogenous compounds.* If nitrogen is present it appears among the products of combustion as the higher oxides of nitrogen and must be removed before pressure measurements are taken. The trap *K* is therefore cooled in liquid air during the combustion and collects all condensable products. After excess oxygen has been pumped away, the condensed gases are brought to laboratory temperature and transferred at a pressure of 5 mm. by way of the heated reduction tube *Cu* to the traps *p* and *o*, cooled as before. This reduces nitrogen oxides to nitrogen which is not condensable and can therefore be pumped away and the water and carbon dioxide measured manometrically in the manner described.

*Quantitative test of method.* The method was tested by the analysis in replicate of a number of pure compounds on both the 1-2- and 2-5-mg. scale. The average deviation from theoretical

was 0.5 and 1.6% for carbon and hydrogen, respectively, on the 1–2-mg. scale, and 0.55 and 1.0% on the 2–5-mg. scale. Neither nitrogen, sulphur, nor chlorine interfered with the accuracy of the determination.

Naughton and Frodyma were concerned only with the quantitative production and measurement of water and carbon dioxide for the purposes of analysis. Obviously, however, these products, if labeled, are available for isotopic assay, and the workers responsible for the method to be described next have introduced the necessary modifications.

*Method of Anderson, Delabarre, and Bothner-By*

These workers (8) have described an apparatus which differs from that of Naughton and Frodyma in several details, but chiefly in being completely demountable by means of standard taper joints. It is shown in Fig. 38.

*Combustion tube.* This is made entirely of quartz and fitted with standard taper joints. It is 13 mm. in outside diameter and about 45 cm. long, its shape depending on whether or not it is to be connected to the nitrogen oxide reducing unit. The filling differs slightly from that used by Naughton and Frodyma and consists of a roll of silver wire (1 cm.), a layer of copper oxide wire (18 cm.), more silver wire (1 cm.), and a roll of platinum screen (2 cm.) to hold the rest of the filling in place. As the combustion tubes are made of quartz they will tolerate a higher temperature than will glass tubes and can therefore be maintained at 750°–800° by means of an electric furnace.

These workers recommend two apparatuses to be used for nitrogenous and non-nitrogenous substances, respectively. For nitrogenous substances the combustion tube has its delivery end co-axial for connection to the nitrogen oxide reducer as shown in the bottom row of components in Fig. 38. For non-nitrogenous substances, the combustion tube is bent at right angles as shown in the top row of components in Fig. 38 and can be connected to the "train by-pass" or to the heavy-water trap which is used when deuterium is to be assayed. No provision is described in the published account for dealing with deuterium-labeled nitrogenous

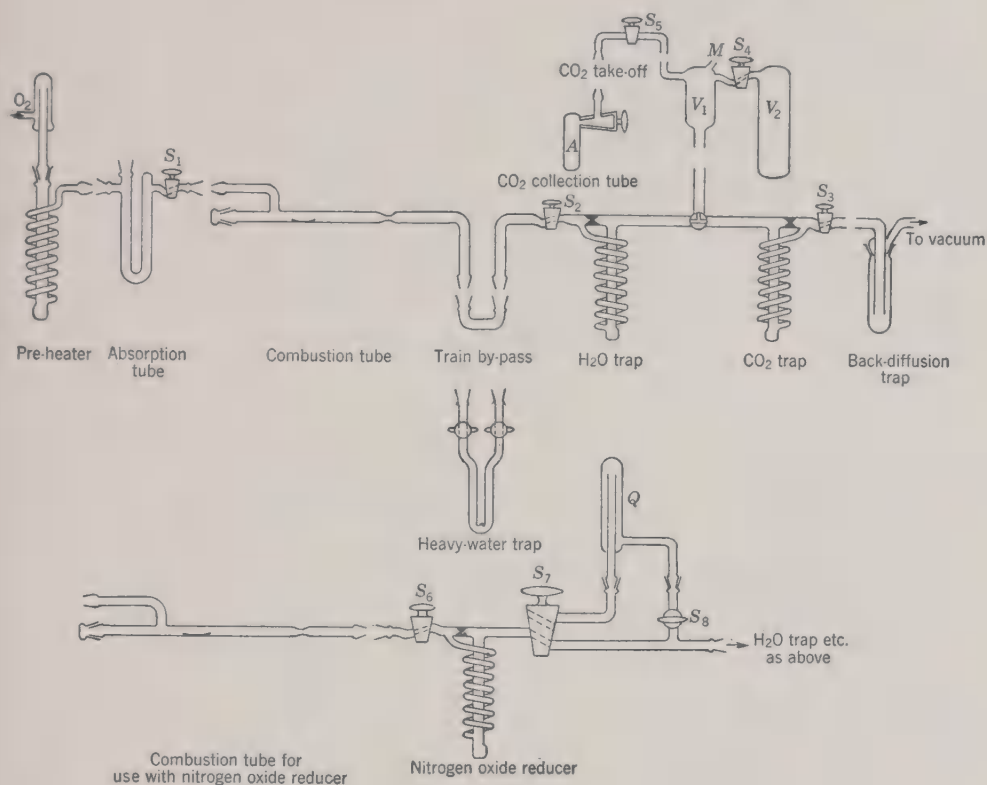


FIG. 38. Demountable combustion apparatus (Anderson, Delabarre, and Bothner-By). Components are shown in order of assembly. If deuterium is to be measured, the *heavy-water trap* is inserted in the position occupied by the *train by-pass*. If the nitrogen oxide reducer  $Q$  is required, the other combustion tube (shown in the lower part of the drawing) is used. The  $CO_2$  collection tube  $A$  is used to collect  $C^{13}O_2$ , prior to mass spectrometric analysis, or  $C^{14}O_2$  for conversion to  $BaCO_3$  for solid counting. If gas counting is to be used, the counter is attached directly to the  $CO_2$  take-off.  $S_1$ – $S_8$ : Stopcocks;  $V_1$  and  $V_2$ : Calibrated volumes, with attachment  $M$  for two-liquid manometer shown in Fig. 11.

compounds but it is obvious that the heavy-water trap could be inserted in the train after the nitrogen oxide reducer by means of suitable adaptors.

*Manometric system.* Products of combustion are measured in the calibrated volumes  $V_1$  and  $V_2$ .  $V_1$  is a bulb 45 mm. in diameter and 80 mm. long, and  $V_2$  is another bulb of the same diameter but twice the length. They are connected by a 2-mm.-bore stopcock



( $S_4$  in Fig. 38).  $V_1$  is connected to the two-liquid manometer already described in detail (page 32) and is provided also with a side arm ( $\text{CO}_2$ -take-off) to which the carbon dioxide collection tube or gas counter can be attached. According to the volume of gas to be measured,  $V_1$  is used alone or with its capacity added to that of  $V_2$ . Greater range in gas measurements could be achieved by increasing the number of bulbs, but these two have proved sufficient for the range 0.3 to 6 mg. carbon, for which the combustion apparatus is designed.

*Oxygen supply.* The oxygen flow is controlled directly by means of the reduction valve on the gas cylinder and by a glass regulator using concentrated sulphuric acid. It is then passed through a drying tower, containing alternate layers of Anhydron and Ascarite separated by glass wool, and through a pre-heater which catalytically oxidizes any combustible impurities. It then passes to the combustion tube through a U-trap absorption tube by means of which any water or carbon dioxide formed in the pre-heater may be removed.

*Procedure; non-nitrogenous compounds.* When the sample contains no nitrogen, the apparatus is assembled in the order shown in the upper portion of Fig. 38. The sample, containing from 0.3 to 6 mg. carbon, is weighed into a platinum boat and introduced into the combustion tube through the stoppered end. The train is exhausted by means of a mechanical pump connected to the opposite end of the apparatus. The ultimate vacuum obtainable on the pump is 0.005 mm. for, as already noted (page 11), these workers do not approve of the higher vacua provided by diffusion pumps. The *water trap* is cooled with solid carbon dioxide and the *carbon dioxide* and *back-diffusion traps* with liquid nitrogen. Then, by opening stopcock  $S_2$  slightly, oxygen is drawn slowly through the pre-heater, absorption tube, and combustion train at such a rate that the pressure in the manometric system rises by 3 cm.

The sample in the combustion tube is heated with a small auxiliary furnace, raising the temperature slowly to  $700^\circ$  and maintaining it there for about 5 min. The stopcock  $S_1$ , admitting oxygen to the system, is closed and stopcock  $S_2$  slowly opened,

thus evacuating the train while maintaining about the same rate of gas flow through the traps. The water and carbon dioxide are then measured by allowing them to evaporate into the calibrated volumes in turn and observing the pressure.

*Nitrogenous compounds.* If the sample contains nitrogen, the resulting oxides of nitrogen must be removed not only because they cause errors in the carbon dioxide measurement but also because they interfere with the operation of gas counters. The second combustion tube and train shown in the lower portion of Fig. 38 is then used. The nitrogen oxide reducer unit consists of a spiral trap connected by stopcocks and standard joints to a quartz section Q which consists essentially of an annular space containing a roll of fine-mesh copper screen freshly reduced with methanol vapor before sealing off in the trap.

In use, the spiral trap of the reducer unit is cooled in liquid air and, after pumping off excess oxygen, the condensed gases diverted through the copper screen, now heated to  $450^{\circ}$ . At this temperature, reduction of nitrogen oxides but not of carbon dioxide occurs; at higher temperatures it was found that some reduction of carbon dioxide resulted, whereas at lower temperatures some nitrogen oxide escaped reduction. After passing through the reducer unit the carbon dioxide and water are trapped and measured in the manner already described.

*Carbon-13.* If  $C^{13}$  is to be assayed, the carbon dioxide, after measurement, is condensed into a previously evacuated carbon dioxide transfer apparatus A (see Fig. 38) attached to  $V_1$  which can then be removed and taken to the mass spectrometer.

*Carbon-14.* If  $C^{14}$  activity is to be measured it may be done either by gas counting or by solid counting as barium carbonate. Two methods of gas counting are recommended by the originators of this apparatus; the proportional counting method of Bernstein and Ballentine (page 66) or the Geiger counting method of Brown and Miller (page 75). In the former case, the measured volume of carbon dioxide is condensed into a gas counter which is then removed and attached to another apparatus for the addition of methane. The Geiger counting method necessitates the admixture with the carbon dioxide of 10% of carbon disulphide

vapor. It would therefore be advantageous if this were introduced into the counter before attaching to the carbon dioxide take-off. Since Geiger counting is best carried out at a standard pressure (22 cm.) it would then be necessary to remove the counter and attach it to a source of inactive carbon dioxide for adjusting the pressure.

*Solid counting.* For solid counting, a piece of apparatus similar to that used for collecting the  $C^{13}O_2$  is attached to the carbon dioxide take-off and the radioactive carbon dioxide condensed into it. It is then removed and connected by means of standard-joint apparatus to a flask containing a saturated solution of barium hydroxide. This flask is evacuated on the filter pump through a two-way stopcock which is then turned to disconnect the vacuum and connect the flask to the carbon dioxide vessel. The barium hydroxide solution is warmed to  $80^\circ$  and carbon dioxide admitted to it. Barium carbonate appears at once and the reaction is complete in 5 min. The precipitate is filtered off by standard procedures and counted.

*Deuterium.* For the determination of deuterium in the combustion water, a heavy-water trap is inserted in the train in the position shown in Fig. 38. It is not now possible to measure the water produced manometrically (although presumably it could still be weighed), but the yield of carbon dioxide trapped in the usual way is available for the purpose of ascertaining that quantitative combustion has occurred. A rather tedious procedure is then carried out. Several unweighed samples of the material to be analyzed are first combusted and the water and carbon dioxide discarded. This is to ensure that the adsorbed water on the glass surfaces (which have been reduced to a minimum by trapping the water vapor as it emerges from the combustion tube) has the same isotopic composition as the combustion water. It is to be noted that if a permanent high-vacuum apparatus, thoroughly exhausted by means of diffusion pumps, is used, this precaution is not necessary (9). A carefully weighed sample is next submitted to combustion, and the trap containing the heavy water removed from the apparatus for separate treatment. Anderson and his colleagues point out that combustions on the scale of 6



mg. carbon or less do not produce enough water for assay by the falling-drop method (for which at least 100 mg. is needed), but they say that the apparatus can be modified for the combustion of larger samples by adding a much larger volume to  $V_1$  and  $V_2$ . This would certainly permit the measurement of much larger samples of carbon dioxide but does not solve the chief problem which, in the present author's experience, lies in ensuring the quantitative combustion of large samples rather than in collecting and measuring the products. Payne, Campbell, and White (10) find that the combustion of 500-mg. samples will proceed smoothly to completion in a stream of oxygen only if the temperature of the sample-heating furnace is raised at a constant rate from room temperature to  $700^\circ$  in the space of about 30 min.

The efficiency of the method on the 6-mg. scale was tested by Anderson and his colleagues by the analysis in replicate of a number of pure compounds. From the published data it appears that the maximum error was about 2% in the carbon and 6% in the hydrogen values. The analysis of two deuterium compounds for carbon and hydrogen was accomplished with about the same accuracy. Deuterium was also determined in duplicate for each compound with a reproductibility of about 6%. It is not stated exactly how the deuterium was determined but it appears that a sufficiently large sample was combusted for the application of the falling-drop method.

## THE PRODUCTION OF LARGE SAMPLES OF COMBUSTION WATER

### *Method of Payne, Campbell, and White*

Payne, Campbell, and White (10), who need relatively large amounts of water for the successful application of their method of tritium determination (page 171), have described a combustion technique designed to deal with up to 600 mg. of organic material. In order to minimize the memory effects due to exchange, they avoid glass and porous materials in the construction of their apparatus. They point out that the copper oxide filling and the quartz or glass tubes used in most combustion trains are porous and that the porosity of quartz increases with use. They



therefore replace the copper oxide recommended by Pregl with platinum gauze, already known from the work of Kopfer (12) and Dennstedt (13) to promote combustion, and make their combustion tubes of metal. Inconel metal is chosen as being resistant to oxidation at high temperatures and because there is reason to

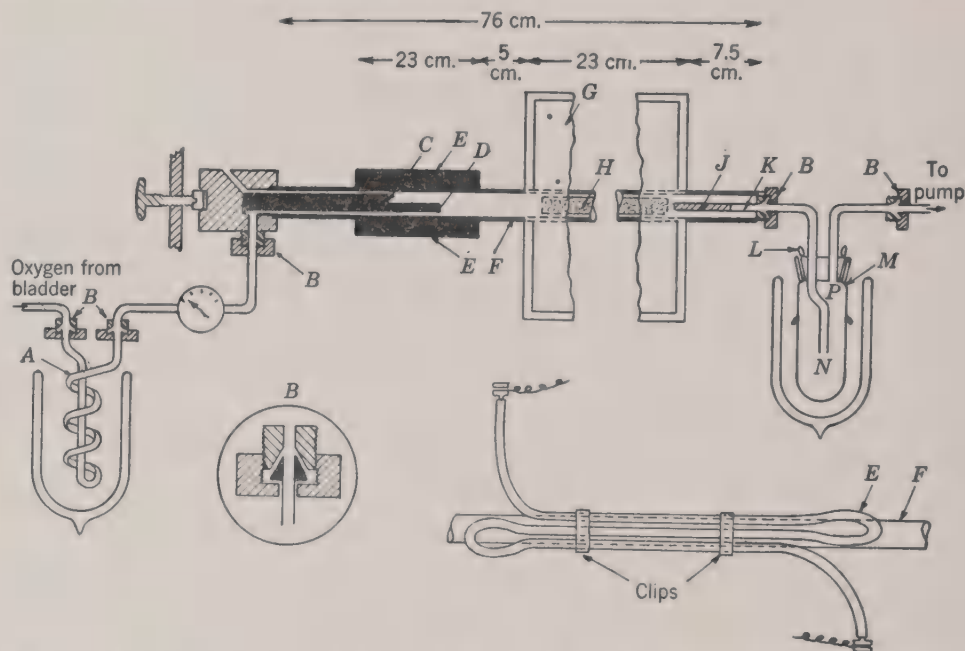


FIG. 39. Combustion apparatus for tritiated samples (Payne, Campbell, and White).

- |   |  |
|---|--|
| A: Copper spiral.   | H: Roll of platinum gauze 20 cm. long.   |
| B: Brass crush nipples.   | J: Copper rod.   |
| C: Sample holder block.   | K: Copper tube.  |
| D: Sample holder.   | L: Brass B29 cone joint.   |
| E: Sample heater.   | M: Pyrex vessel.   |
| F: Combustion tube; inconel, $\frac{3}{8}$ in. inside diameter, $\frac{5}{8}$ in. outside diameter. | N and P: Copper tubes to which chemical trap can be attached instead of cold trap. |
| G: Main furnace at $1000^{\circ}$ .   |  |

believe that high-nickel alloys are less liable to contamination with tritium than are other metals.

*Combustion tube.* The inconel combustion tube (see Fig. 39) is 76 cm. long and 9.5 mm. in internal diameter. It contains a roll of 150-mesh platinum gauze *H* 20 cm. long which is maintained

at  $1000^{\circ}$  by the main furnace. This temperature, higher than normally used for microcombustions ensures complete oxidation and reduces catalyst poisoning. The copper rod *J* which lies next to the platinum gauze in the delivery end of the combustion tube gets sufficiently hot to prevent condensation of water before it reaches the trap.

*Sample holder and sample-heating furnace.* The sample contained in a silica boat is introduced through a gas-tight breech and mounted on the holder *D*. This fits loosely in the combustion tube, leaving a small annular space where, during a combustion, there is therefore a high linear gas velocity which prevents back diffusion of volatile compounds. Payne, Campbell, and White have found that the combustion of large samples proceeds smoothly to completion only if heated very slowly. The temperature of the electric sample-heating furnace is therefore raised at a controlled rate by means of a constant-energy-control device (Simmerstat) driven by an electric clock. This raises the temperature from that of the laboratory to  $700^{\circ}$  during the space of 30–45 min. In an oxygen stream of 20–50 ml./min., even volatile substances burn smoothly by this procedure. Since each sample must be inserted when the furnace is cool, long delays between combustions are avoided by using a pair of unlagged heaters *E*, each consisting of a 700-watt element supported in magnesia contained in an inconel sheath. The heaters are folded and clipped to the combustion tube as shown in the figure. When the current is switched off at the end of a combustion, these heaters are found to cool within a few minutes.

*Oxygen supply.* Oxygen is stored in a 20-liter rubber bladder and is drawn through the apparatus by a small mechanical pump. Transient variations in the oxygen flow are prevented by the insertion of a 5-liter metal reservoir between the pump and the apparatus. The rate of flow of oxygen is controlled by a valve which permits air to leak into the reservoir. This arrangement results in a steady flow of oxygen and eliminates the necessity for the Mariotte bottles recommended by Pregl. The same result can be obtained somewhat more simply by the use of a "control" stop-cock described in Chapter II (Fig. 6).

The oxygen is purified before it enters the combustion tube by passage through a copper spiral trap *A* cooled in a solid carbon dioxide bath. This eliminates only water and such other impurities as are condensed at this temperature. If the collection of combustion carbon dioxide as well as water were required, this trap would need to be cooled in liquid oxygen to remove any contaminating carbon dioxide.

*Collection of combustion water.* Water is stripped from the oxygen stream by means of the concentric-type trap *M* which is cooled in a solid carbon dioxide bath. The brass-to-glass joint *L* on this trap is lubricated with silicone vacuum grease because it gets hot by conduction from the furnace. If the tritium content of the water is to be determined by the method devised by these authors (11), the trap *M* is detached from the combustion train and connected to the tritio-methane generating apparatus at *B* in Fig. 60 (page 172) by means of the adaptor shown in Fig. 40.

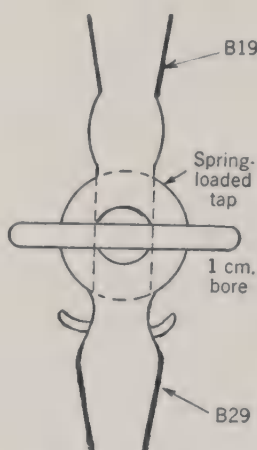


FIG. 40. Adapter for connecting glass vessel *M* (in Fig. 39) to tritium determination apparatus at *B* in Fig. 60.

*Test of method.* When only a chilled trap was used for stripping the combustion water from the gas stream, Payne, Campbell, and White found that 90–95% of the expected water yield was collected. They point out that, for specific activity measurements, quantitative collection is unnecessary and that this yield of water is therefore satisfactory. But since quantitative combustion is desirable if isotopic fractionation is to be avoided, it was tested for by replacing the cold trap *M* by chemical traps. All the water formed could then be collected and weighed. The results then obtained from the combustion of a number of test substances indicated that the error never exceeded 2%.

Possible contamination of the apparatus was tested for by the combustion of a tritiated test substance containing about  $0.1 \mu\text{c.}$ , followed by flushing of the apparatus at the rate of 30 ml./min. for 15 min. The combustion water then obtained from a sample

of inactive dextrose was found to contain only 0.4% of the activity originally in the radioactive test substance. This is a very low order of contamination.

The other possible source of error tested for by the designers of this method was interference by nitrogen and sulphur. On combustion, these elements give rise to oxides which combine with water. It was therefore necessary to ascertain that they would not do so selectively, thus altering its isotopic composition. A mixture of the test substance with 1-amino-2-naphthol-4-sulphonic acid was therefore combusted and the combustion water assayed. It was found to have substantially the calculated value, showing that neither nitrogen nor sulphur interfere.

#### REFERENCES

1. W. D. ARMSTRONG, L. SINGER, S. H. ZBARSKY, and B. DUNSHEE, *Science* **112**, 531 (1950).
2. D. D. VAN SLYKE and J. M. NEILL, *J. Biol. Chem.* **61**, 523 (1924).
3. D. D. VAN SLYKE and J. FOLCH, *J. Biol. Chem.* **136**, 509 (1940).  
D. D. VAN SLYKE, J. PLAZIN, and J. R. WEISIGER, *J. Biol. Chem.* **191**, 299 (1951).  
D. D. VAN SLYKE, R. STEELE, and J. PLAZIN, *J. Biol. Chem.* **192**, 769 (1951).
4. A. LINDENBAUM, J. SCHUBERT, and W. D. ARMSTRONG, *Anal. Chem.* **20**, 1120 (1948).
5. H. ROTH, "Quantitative Organic Microanalysis of Fritz Pregl," 3rd English Ed. J. & A. Churchill Ltd. London, 1937.
6. J. J. NAUGHTON and M. M. FRODYMA, *Anal. Chem.* **22**, 711 (1950).
7. J. B. NIEDERL and V. NIEDERL, "Organic Quantitative Microanalysis." John Wiley and Sons Inc., New York; Chapman and Hall Ltd., London, 1942.
8. R. C. ANDERSON, Y. DELABARRE, and A. A. BOTHNER-BY, *Anal. Chem.* **24**, 1298 (1952).
9. W. G. DUNCOMBE and R. F. GLASCOCK, unpublished.
10. P. R. PAYNE, I. G. CAMPBELL, and D. F. WHITE, *Biochem. J.* **50**, 500 (1952).
11. D. F. WHITE, I. G. CAMPBELL, and P. R. PAYNE, *Nature* **166**, 628 (1950).
12. F. KOPFER, *Z. anal. Chem* **17**, 32 (1878).
13. M. DENNSTEDT, *Ber. Deut. chem. Ges.* **30**, 1590 (1897).



## CHAPTER VI

### COMBUSTION OF SAMPLES CONTAINING DEUTERIUM, TRITIUM, $C^{13}$ , AND $C^{14}$ , USING PERMANENT VACUUM LINE

This technique, essentially that described by the present author in 1952 (1), is similar in principle to several of those described in Chapter V but the apparatus is designed for use in conjunction with the permanent high-vacuum line as shown in Fig. 43. Incorporation of the combustion train in the vacuum apparatus is a considerable advantage because the radioactive combustion water and carbon dioxide then become immediately available in a convenient place for the next stage in their isotopic analysis; neither of them need be condensed into a separate vessel and detached from the apparatus for assay elsewhere. This principle could be usefully extended to the assay of  $C^{13}$  by building the vacuum line adjacent to, or even connected into, that of the mass spectrometer. Where this is not possible, as in the present author's laboratory, the samples of  $C^{13}O_2$  must be sealed off in a break-seal tube and removed from the apparatus. The advantages of this arrangement for  $C^{14}$  and tritium assay, however, are still considerable.

Provision is made in this technique for division of each radioactive gas sample into several separate portions for replicate counting. This procedure also has several advantages. First, since high counting rates are undesirable in Geiger counters owing to dead-time coincidence losses, it is best if a sample containing an activity of between 1000 and 2000 counts/min. is taken. Second, the counting of two or more separate and differently sized portions of the gas provides a means of cross-checking the specific activity measurements and reduces the risk of spoiling the whole assay by one erroneous measurement.

Instruments for measuring water vapor are not included in

the apparatus although a two-liquid manometer could obviously be added if necessary. However, although quantitative combustion and collection is important, the chief purpose of the technique is isotopic assay. For that purpose the measurement of water as such is seldom required, the yield of carbon dioxide being taken as the criterion of completeness of combustion. This is justified by the finding (2) that when the samples combusted are large enough for the resultant water to be weighed accurately, the yield is often quantitative under conditions in which the yield of carbon dioxide is not. The total carbon dioxide and portions of it greater than 1 standard ml. are measured in a manometric chamber connected to a simple manometer. Portions of it less than 1 ml. and down to about 10  $\mu$ l. in volume are measured in a McLeod gauge. The construction and method of operating this instrument has already been described.

*Scale.* The method, like several others already described in Chapter V, is designed for use on the 5- to 10-mg. scale. This weight of most organic substances yields about 10 ml. carbon dioxide and 10 mg. water; 10 ml. carbon dioxide is usually more than sufficient for the counting of several portions and some of it can be spared for the mass spectrometric determination of  $C^{13}$  if required. Similarly, 10 mg. water is sufficient for conversion to hydrogen by the method of Graff and Rittenberg (page 147) or to butane by the present author's own method (Chapter IX) if deuterium or tritium is to be determined. Thus, unless the falling-drop method, which requires about 100 mg. water, is to be applied to deuterium assay, this scale is particularly useful both because of the convenient volume of gaseous products and because about 10 mg. of any particular metabolite is often as much as can be isolated in a biochemical experiment. Three isotopes ( $C^{13}$ ,  $C^{14}$  and D, or T) could therefore be determined in the products of a single combustion.

## APPARATUS

### *Furnace Construction*

In most papers cited, including those of the present author, no instructions are given for making the furnace. Some authors

give the names of manufacturers of commercial models but, for readers who prefer to have their furnace made in their own workshops, the following instructions may be useful.

The heating elements should not be wound directly on the quartz combustion tube, especially if it is to be filled with copper oxide. This substance vigorously attacks quartz at temperatures above about  $850^{\circ}$ , and any local hot spots may result in serious damage. It is better to surround the combustion tube with a separate cylindrical heating furnace which can be made of either quartz or metal tubing. Quartz furnaces are less liable than metal ones to burn out through short circuiting of the elements but are more likely to get broken by rough handling. The present author has had satisfactory service from both kinds.

*Quartz furnace.* Quartz tubes about 1 ft. long and 1 in. in diameter are available commercially (3), either plain or with a spiral groove of ten turns to the inch already cut to take the heating element. If the tube is not grooved, the element must be wound on asbestos paper, as described for metal furnaces, but the grooved tubes are much more convenient and should be obtained if possible. A tube 12 in. long with a spiral groove cut over 10 in. of its length takes about 26 ft. of wire. A moderately well-lagged furnace consuming about 250 watts will maintain the combustion tube at  $700^{\circ}$ , which is about the maximum temperature likely to be needed. It is convenient, however, if an element consuming about 500 watts at full power-line input is used and the input reduced and adjusted by means of a variable transformer or constant-energy control (Simmerstat). Resistances of 25 ohms for 110-volt lines or 100 ohms for 250-volt lines will thus be needed and nickel-chromium resistance wire of such a gauge that this resistance is provided in about 26 ft. should therefore be chosen.

The wire is wound firmly into the spiral groove and the ends anchored in  $\frac{1}{2}$ -in.-wide copper straps bolted around the tube as shown in Fig. 41. The element is lagged with about half an inch thickness of asbestos string and finished neatly with a layer of glass tape, the free end of which is stuck down with a smear of sodium silicate. The glass tape should not be wound on, however,



until the furnace has been heated to a sufficient temperature to burn away organic matter in the string because the smoke produced causes unsightly discoloration.

*Metal furnace.* A satisfactory furnace can be wound on metal tubing if care is taken to prevent the wire from short circuiting on it. A stainless steel tube 1 ft. long and 1 in. in diameter is best, although mild steel can also be used. It is first covered with two layers of  $\frac{1}{16}$ -in.-thick, wet asbestos paper which should extend at each end about 1 in. beyond the intended extremities of the element. As a precaution against attack of the element by chemicals in the paper and as a further measure of electrical insulation, a layer of glass tape is wound over the wet paper. The heating element is wound on tightly while the underlying paper is still

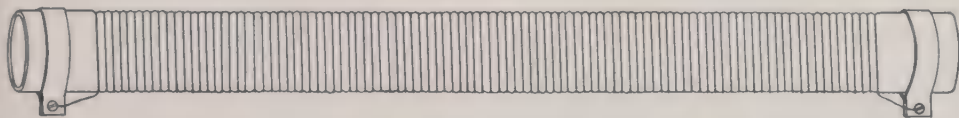


FIG. 41. Quartz tube furnace, showing grooves, winding, and copper terminal straps.

damp, a grooved bed thus being formed. The wire is anchored at each end on copper straps bolted in place over the asbestos paper left for this purpose. A layer of glass tape is wound tightly over the element to keep it in place and to hinder movement and possible short circuiting between turns when the furnace is in service. The lagging is then completed with asbestos string and glass tape as already described for the quartz furnace. The furnace is dried out under its own power but it is advisable to do so slowly with a low current input to avoid short circuiting through the wet asbestos.

*Temperature adjustment.* The appropriate setting of the input controls is found by means of an empty combustion tube clamped in position and containing a thermocouple at about its mid-point. Quartz wool plugs are inserted between the combustion tube and furnace at each end to prevent loss of heat by convection and the electrical controls adjusted to give a steady temperature. If the



thermocouple is made of platinum/platinum-rhodium (13% rhodium) a temperature of  $700^{\circ}$  produces an e.m.f. of 6.72 mv.

### *Combustion Train*

*Combustion tube.* This is made from quartz and is 55 cm. long with an internal diameter of 10 mm. It is fitted with standard taper joints as shown in Fig. 42. The 30 cm. of its length within

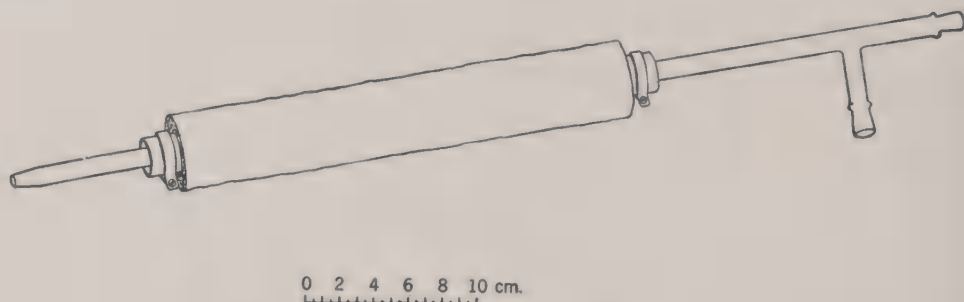


FIG. 42. Combustion tube and furnace.

the furnace is packed with standard microanalytical grade copper oxide (from wire) kept in place with plugs of quartz fiber. It has been found that the plug nearest the sample being combusted must be within the hot zone of the furnace otherwise it may collect smoke which will then escape combustion. This is not only undesirable because of possible isotopic fractionation but because of the danger of contaminating later samples.

No substance for the absorption of nitrogen oxides is included in the filling since, together with traces of tritium activity which may escape into the carbon dioxide, it is subsequently eliminated by treatment of the gas with hot copper and active charcoal.

*Traps.* The combustion tube is connected directly into the high-vacuum apparatus by means of a standard taper joint to the right of stopcock  $S_{28}$  in Fig. 43. This stopcock and joint, being near the furnace and therefore liable to get hot, are lubricated with silicone high-vacuum grease. It is useful to have a connection to rough vacuum (not shown in the figure) into the U-trap  $T_6$  just to the right of stopcock  $S_{23}$  since the rough vacuum pump is suitable for drawing the oxygen through the train. This arrangement makes it possible to avoid interference with other

operations on the vacuum line caused by the passage of gas down the mains.

Trap  $T_7$  is spiral and contains 1 meter of 6-mm. tubing. It has been found that neither the spiral trap alone nor two U-traps

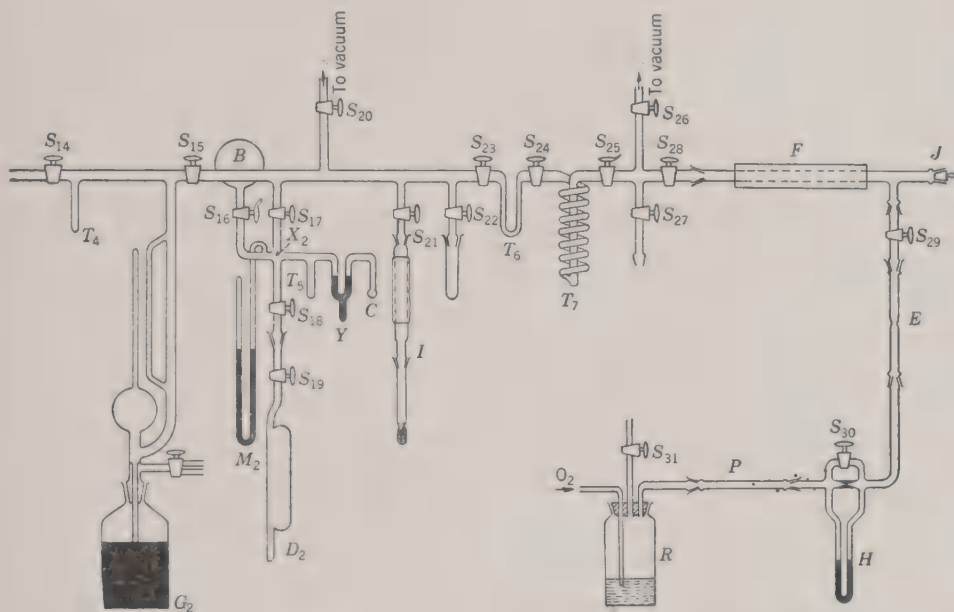


FIG. 43. Vacuum-line combustion train for the combustion of 10-mg. samples and the assay of  $C^{13}$ ,  $C^{14}$ , and tritium (part of main apparatus shown in Fig. 1).

$B$ :  $CO_2$  reservoir.

$C$ :  $CS_2$  reservoir.

$D_2$ : Gas counter.

$E$ : Oxygen purification tube.

$F$ : Furnace and combustion tube.

$G_2$ : McLeod gauge.

$H$ : Flowmeter.

$I$ : Active charcoal tube and nitrogen oxide reducer (see Fig. 45).

$J$ : Joint and stopper (B14).

$M_2$ : Manometer.

$P$ : Platinized asbestos tube.

$R$ : Bubbler and oxygen reservoir.

$S_{14}$ – $S_{31}$ : Stopcocks.

$T_4$ – $T_7$ : Traps and condensation tubes.

$X_2$ : Manometric chamber.

$Y$ : Mercury cut-off.

such as  $T_6$  will achieve quantitative stripping of carbon dioxide from the oxygen stream. This may be due to the gas phase condensation observed by Buchanan and Nakao (page 21). The few per cent that escapes condensation in the spiral trap, however, is

retained by the U-trap. The side arm that contains stopcock  $S_{22}$  is available for storing samples of gas awaiting assay, and it has been found useful to increase the length of tubing between  $S_{15}$  and  $S_{23}$  and to attach more side arms. To one of them, as will be described later, is attached the nitrogen oxide reducer and active charcoal tube.

*Oxygen supply.* A supply of oxygen at atmospheric pressure is conveniently obtained from a gas cylinder by bubbling it through concentrated sulphuric acid in the reservoir  $R$  and allowing it to run to waste through stopcock  $S_{31}$ . It is drawn from the reservoir into the combustion train by operating the control stopcock  $S_{25}$  with the apparatus to the left of it on open pumps. The difficulty of trapping a condensable component from a gas stream at atmospheric pressure with liquid air-chilled traps has already been referred to (page 21). The system of traps described above will not remove the carbon dioxide and water quantitatively from the oxygen unless the pressure is 2 mm. or less. Thus if it is desired to draw the oxygen through the train by means of the rough vacuum pump as suggested above, it is necessary to ascertain beforehand that the pump is capable of keeping the pressure in the traps low enough when the oxygen is passing at the prescribed rate of 50 ml./min.

On the way to the combustion tube the oxygen passes through a heated quartz tube  $P$  (15 cm. long, 10 mm. diameter) which contains platinized asbestos to oxidize combustible impurities; through a simple flowmeter  $H$ ; and finally through an absorption tube packed half with soda asbestos and half with anhydrous magnesium perchlorate. It is to be noted that this material can become saturated with water without showing any marked change in appearance and the packing should therefore be regenerated from time to time by heating to about  $200^{\circ}$  under reduced pressure (filter pump). This will be necessary more frequently if the flowmeter contains an aqueous solution. The standard taper joints on the catalyst tube  $P$  and on the lower side of the absorption tube  $E$  can safely be left dry. Stopcock  $S_{29}$  and the joints on either side of it are lubricated with silicone grease because of their proximity to the hot furnace.

*Flowmeter.* Of several satisfactory designs in current use, one is illustrated in Fig. 44. It is made by constricting a piece of glass tubing so that when gas is flowing through it at a suitable rate there is an appreciable difference in pressure across the constriction. This difference is measured by means of a simple manometer.

Several attempts will probably have to be made before a constriction of a suitable size is obtained and its actual size will depend on the manometric fluid to be used. If mercury is chosen, a larger pressure difference and hence a smaller opening to the constriction will be needed to give a convenient reading (say 5 cm. for a flow of 50 ml./min.) than if colored water or oil is used. If the manometer contains mercury then the combustion will proceed at a pressure somewhat less than atmospheric although there appears to be no disadvantage in this. If colored water is used there is a greater danger of drawing it up into the drying tube *E* by carelessly opening the control stopcock. This may be guarded against by inserting a stopcock-controlled bypass over the constriction as shown in Fig. 44. The bypass is also kept open when the combustion tube is not in use and a rapid flow of oxygen is being passed through it as a precaution against memory effects; it is closed only when a combustion is about to begin.

The flowmeter is calibrated on the apparatus by disconnecting the oxygen reservoir and attaching an empty laboratory wash bottle of about 1 liter capacity. It is arranged so that as air is withdrawn water is drawn into it from a measuring cylinder. The time taken for a known amount of water to be withdrawn from the measuring cylinder, and the corresponding reading on

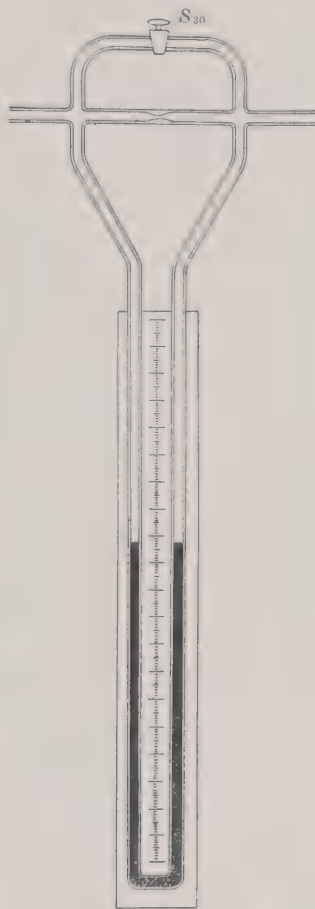


FIG. 44. Flowmeter.



the flowmeter, is recorded over a suitable range of flow rates obtained by adjusting the control stopcock  $S_{25}$ .

*Manometric system.* The manometric chamber  $X_2$  is bounded by stopcocks  $S_{16}$ ,  $S_{17}$ ,  $S_{18}$ , the manometer  $M_2$ , and the mercury cut-off. It has already been described in detail on page 83 and Fig. 29 and is shown in relation to the combustion apparatus in Fig. 43. Samples of gas condensed in the manometric chamber are allowed to warm to laboratory temperature and are measured by observing the pressure registered on the manometer  $M_2$ . The volume in standard milliliters is then obtained by reference to a calibration curve. Samples smaller than 1 ml. are measured in the McLeod gauge  $G_2$  as already described.

#### *Procedure with New Apparatus*

*Cleaning.* The heaters on the furnace having been switched on long enough for the temperature to have reached a steady  $700^\circ$ , the oxygen supply to the reservoir  $R$  is started up so that it is bubbling freely, although without excessive splashing, through the sulphuric acid and issuing from the stopcock  $S_{31}$ . The stopcock  $S_{30}$  on the flowmeter bypass is opened and stopcock  $S_{31}$ , through which the oxygen runs to waste, is closed so that the oxygen is diverted through the still-open standard taper joint  $J$ . As much as possible of that part of the combustion tube lying outside the furnace is heated to redness with the hand torch over a period of several minutes to burn away traces of organic matter. The oxygen is then diverted through the combustion tube by closing  $J$ , opening stopcocks  $S_{27}$  and  $S_{28}$ , and allowing it to pass through the tube for about an hour. This procedure is carried out between combustions or at any time when the furnace is temporarily out of use.

*Blanks.* Stopcock  $S_{31}$  on the oxygen reservoir is opened again and  $S_{27}$  closed. The apparatus to the left of the control stopcock  $S_{25}$  is opened to the pumps, and the traps are heated with a soft flame and then allowed to cool. They are then both chilled in liquid air. The flowmeter bypass stopcock,  $S_{30}$ , is closed and the control stopcock  $S_{25}$  cautiously opened until the flowmeter registers a rate of flow of 50 ml./min. The part of the combustion tube

outside the furnace is heated to redness over a period of 5 min., and the combustion tube then completely evacuated by closing  $S_{29}$  and gradually opening the control stopcock.

If the train contained any combustible matter the resultant carbon dioxide and water will now be in the traps. They are therefore isolated by closing stopcocks  $S_{23}$  and  $S_{25}$  and the contents, if any, combined by removing the liquid air from around the spiral trap and allowing it to warm up. The amount of carbon dioxide present can be found by subliming it into the manometric chamber and observing the pressure exerted as described below. Its absence is usually a sufficient indication that the combustion train is free from organic matter. If any measurable amount is found, a leak in the apparatus should first be suspected since laboratory air may contain abnormally high concentrations of carbon dioxide when the solid gas is in use as a coolant.

*Quantitative combustion.* It must next be ascertained that organic substances can be combusted quantitatively. For this purpose such pure chemical substances as seem best to the operator may be chosen, but it is worth while to include glucose, as one of the easiest, and a long-chain fatty acid as one of the most difficult substances to combust quantitatively.

As already explained, the 10-mg. scale is the most convenient for this equipment although the present author has obtained quantitative results from as little as 1 mg. About 10 mg. of the pure organic compound is therefore weighed accurately into a clean porcelain boat and introduced through the joint  $J$  (Fig. 43) into the combustion tube about midway between the side arm and the furnace.  $J$  is then closed with a stopper using silicone high-vacuum grease as a lubricant. By manipulating the control stopcock  $S_{25}$  as already described, oxygen is then drawn through the combustion train and traps at a rate of about 50 ml./min. The sample is heated gently until it inflames; when this happens the reading on the flowmeter will drop, sometimes to zero. If it falls below zero, a back pressure is indicated and a loss of combustion products into the absorption tube  $E$  is probable. It can be avoided by using a somewhat higher oxygen flow. The initial drop in the flowmeter reading is usually followed by a rise after

which it drops back to its original value. The combustion is now virtually complete. The soft flame is replaced by a Meker burner and the tube heated to redness for an additional 4 min., stopcock  $S_{29}$  is closed, thus shutting off the oxygen supply, and the combustion tube is evacuated by gradually opening the control stopcock  $S_{25}$ . If the rough vacuum pump is being used for drawing the oxygen through the train, as suggested earlier, the evacuation of the system must now be completed by means of the high vacuum line. Stopcocks  $S_{23}$ ,  $S_{25}$ , and  $S_{28}$  are then closed. All but a few per cent of the water and carbon dioxide which have been formed are now in the spiral trap, the remainder being in the U-trap. The contents of the traps are combined in the U-trap by allowing the spiral trap to warm to laboratory temperature, and stopcock  $S_{24}$  is closed. The liquid air around the U-trap is replaced by a solid carbon dioxide bath and the carbon dioxide in the trap separated from the water by subliming it away into the manometric chamber for measurement.

As already explained, the water is not measured, the yield of carbon dioxide being taken as the criterion of completeness of combustion. Failure to achieve quantitative yields may indicate that the furnace temperature is too low, that the quartz fiber plug holding the filling in place is not hot enough and is retaining uncombusted carbon, or that the oxygen flow is not adjusted to the optimum rate.

The method has been tested using pure glucose and palmitic and stearic acids. Quantities of these substances ranging from 5.8 to 13.4 mg. weighed on an air-damped aperiodic balance (1 division = 0.2 mg.) gave carbon dioxide yields within 1.5% of theoretical. Part of this error may be due to the balance, on which samples of this size could not be weighed with very great precision. The fact that the glucose samples were found to give exactly the theoretical yield of carbon dioxide, whereas the fatty acids yielded too little by up to 1.5% (11.8 ml. instead of 12.00), suggests that absolutely quantitative combustion and collection is not always achieved. The accuracy is quite tolerable, however, for the purposes of isotopic assay. The same order of reproducibility was obtained from the replicate combustion of substances



of unknown composition such as the calcium salts of mixed fatty acids.

### *Procedure with Radioactive Samples*

The sample should always be weighed accurately since if it is a pure compound the yield of carbon dioxide provides an interim check on completeness of combustion and if it is of unknown composition it may be useful to have a record of its carbon content. While the combustion is in progress, a counter, whose background counting rate has been previously determined, is attached to the joint below stopcock  $S_{18}$  and thoroughly evacuated. If the main vacuum line is being used to draw oxygen through the train, however, evacuation of the counter will have to be deferred until combustion is complete.

The evacuated counter is charged with carbon disulphide to a pressure of 2 cm. Stopcock  $S_{17}$  is closed, the carbon disulphide reservoir  $C$  chilled in liquid air, and the mercury cut-off opened. The carbon disulphide is allowed to warm up until the manometer  $M_2$  registers a pressure of 2 cm. The stopcock  $S_{19}$  on the counter is closed, the excess carbon disulphide condensed back into its reservoir, and the mercury cut-off closed.

When the combustion of the sample is complete, stopcock  $S_{29}$  is closed, the train evacuated on the main pumps, and the products of combustion combined in the U-trap  $T_6$ . The carbon dioxide is sublimed off at  $-78^\circ$  into the manometric chamber, warmed to room temperature, and its volume measured. A portion of it must now be introduced into the counter. If its specific activity is such that a few milliliters will give a convenient counting rate, division of the sample at this stage may often be conveniently done by allowing it to expand into the line ( $S_{15}$ – $S_{23}$ ). The pressure will then drop to a few centimeters. Stopcock  $S_{17}$  is closed and the excess carbon dioxide in the line is stored in a side arm. The reading on the manometer is noted and stopcocks  $S_{18}$  and  $S_{19}$  opened, thus allowing the gas to expand into the counter. The pressure of gas is then adjusted with inactive carbon dioxide from the reservoir  $B$  to the value which a counter full of gas at 22 cm. pressure has been found to exert when expanded



into the manometric chamber. (See page 85.) This pressure is usually from 15 to 16 cm. The whole of the gas is then condensed into the counter, stopcocks  $S_{18}$  and  $S_{19}$  are closed, and the counter is detached from the apparatus. After warming up the condensation tube, the outside of the counter is dried and connected to the electronic apparatus for counting.

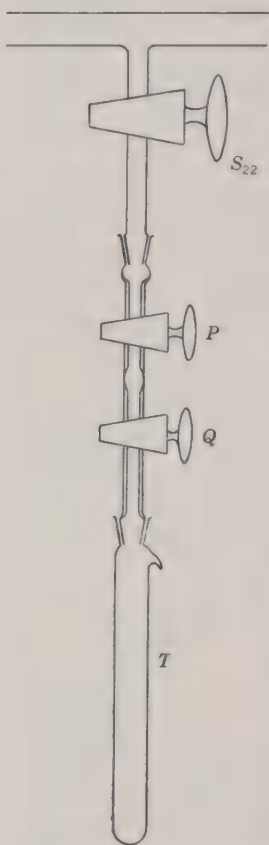


FIG. 45. Gas sample "doser" (see text).

If the specific activity of the gas is greater than about 2500 counts/min./ml., and the volume required to give a convenient counting rate is therefore 1 ml. or less, it is measured in the McLeod gauge  $G_2$ . For this purpose, small fractions of the main sample are conveniently obtained by means of the "doser" illustrated in Fig. 45. It consists of a pair of capillary stopcocks  $P$  and  $Q$  fitted with standard taper joints, by means of which it is connected to the main apparatus and to a tube  $T$  of about 10 ml. capacity into which the gas is condensed. The space between the stopcocks is about 0.5 ml., which allows about one-twentieth of the whole gas sample to be taken at a time and transferred to the McLeod gauge. After being measured it is transferred to the counter and the pressure adjusted as described above.

The combustion water remaining in the U-trap is either discarded if unlabeled or used for the determination of deuterium (page 134) or tritium. If tritium is to be determined by the tritio-butane method described in Chapter IX, the water is transferred down the line to a previously prepared tube of dry butyl magnesium bromide. The butane produced on heating is purified by distillation through a trap cooled in a solid carbon dioxide bath and counted in a stainless-steel-cathode gas counter, the procedure being very similar to that used for the counting of carbon dioxide outlined above.

If  $C^{13}$  is also present, a small portion of the carbon dioxide

(about 2 ml.) is transferred to a break-seal tube and sent to the mass spectrometer for assay. In this connection it should be noted that if the  $C^{14}$  activity in the sample is expected to be low, and both isotopes are to be determined, it may be an advantage to increase the weight of sample combusted to about 15 mg. so that more carbon dioxide is available.

*Purification of carbon dioxide from tritium contamination.* When the sample being combusted contains an appreciable amount of tritium, enough may escape into the carbon dioxide to give it a spuriously high activity. If the combustion tube is properly packed and the temperature correctly adjusted, this contamination is negligible except in those cases where very much more tritium than  $C^{14}$  is contained in the sample. Contamination may then cause a significant error. It is probably due to the presence of minute amounts of volatile organic compounds which are formed when the sample is heated but which escape complete oxidation. The contamination can be completely removed from the carbon dioxide by adsorption on active charcoal.

The charcoal tube is 15 cm. long and 10 mm. in diameter, slightly constricted about 4 cm. from the bottom and bearing a standard taper socket. It contains about 0.5 gm. granular charcoal kept in place with a plug of glass wool inserted below the constriction. This is necessary to prevent the charcoal from being accidentally sucked into the vacuum line during outgassing. The tube is attached to one of the side arms to the left of stopcock  $S_{23}$  (Fig. 43). It is cautiously evacuated and the charcoal finally outgassed by heating to about  $400^\circ$  with a soft flame.

After being measured, the carbon dioxide from doubly-labeled samples is condensed into the tube by means of liquid air which is then replaced by a bath of melting ice. At this temperature the carbon dioxide is desorbed very quickly (half-period about 10 sec.) and quantitative recovery is achieved in 2–3 min. The gas is collected in the manometric chamber for remeasurement and then stored in a side arm until required for counting.

The efficacy of this method has been tested by combusting a sample of sodium 2-tritioacetate,  $CH_2TCOONa$ , containing a total activity of  $1.13 \mu c$ . Before treatment with charcoal, the car-

bon dioxide was found to be contaminated with about 0.1% of the tritium originally present in the sample, but after treatment it was quite inactive. Treatment of the carbon dioxide with charcoal is therefore recommended for all assays of doubly-labeled compounds.

*Procedure with nitrogenous compounds.* The carbon dioxide obtained on combustion of nitrogenous compounds contains oxides of nitrogen. These not only attack the mercury in the manometers and dilute the carbon dioxide but they also interfere with the operation of gas counters. According to Bradley, Holloway, and McFarlane (4), as little as 1 part of nitrogen peroxide in 10,000 of carbon dioxide interferes with the operation of proportional gas counters, causing a loss of counts. In the present author's experience, carbon dioxide-carbon disulphide filled counters operated in the Geiger region according to the method of Brown and Miller (see Chapter IV) are much less sensitive to the presence of the oxides of nitrogen and will tolerate between 0.1 and 0.25% without loss of efficiency. For either counting method, however, these oxides must be eliminated if reliable results are to be obtained.

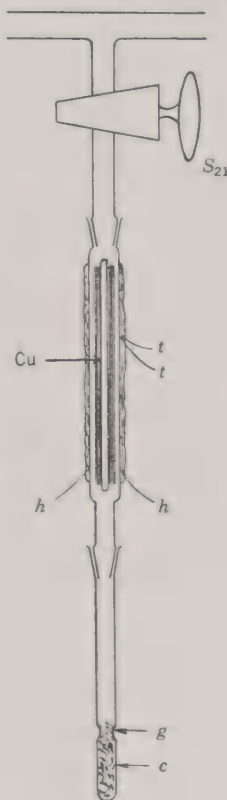
The treatment with active charcoal recommended above for the elimination of tritium activity has been found to reduce the concentration of nitrogen oxides to a level too low for interference with Geiger counting. Unfortunately, not all samples of activated charcoal are equally efficient for this purpose, and a nitrogen oxide reducer containing hot copper is therefore recommended for routine use. This can be combined in a single unit with the charcoal tube used to eliminate traces of tritium and is illustrated in Fig. 46.

The tube is made of Pyrex or quartz and is 15 cm. long and 12 mm. in diameter. Standard taper joints are sealed at each end. A single layer of wet asbestos paper is applied to the tube, and a heating element of resistance about 12 ohms per yard is wound on tightly, being held in position with glass tape exactly as described for the combustion furnace (page 118). A typical reducer used in the author's laboratory is fitted with an element with a total resistance of 28 ohms, requiring an input of 40 volts to maintain the tube at its operating temperature of 450°. After

the element has been wound on the tube it is run hot for a short while to dry out the asbestos and burn away organic matter and is then lagged with several layers of glass tape. A thermocouple buried in the lagging is very useful for the purpose of relating temperature to input voltage.

Into the tube is put a tight roll of bright copper gauze wound on a 3-4 mm. copper rod as a former. The copper rod is left in

FIG. 46. Active-charcoal tube and nitrogen oxide reducer. *c*: active charcoal; *g*: plug of glass wool; *h-h*: heater; *t-t*: thermocouple; *Cu*: Copper gauze; *S*<sub>21</sub>: stopcock on main apparatus (see Fig. 43).



position and is prevented from falling out of the tube by a button previously hammered on its upper end.

The reducer and active charcoal tube (Fig. 46) are attached to the apparatus below stopcock *S*<sub>21</sub>. The two treatments can now be combined in a single operation. In order to avoid attack of mercury in the manometer by oxides of nitrogen, the carbon dioxide from nitrogen-containing compounds is sublimed straight from the U-trap *T*<sub>6</sub> through the hot reducer into the active charcoal tube which is cooled in liquid air. The gas is then desorbed at 0° and passed back through the reducer into the manometric



chamber where it is condensed. Any incondensable gas is pumped away before the carbon dioxide is allowed to warm up for measurement. If much incondensable gas, arising from much oxides of nitrogen, is present it may be necessary to recover the carbon dioxide by passing through a liquid air-chilled U-trap.

*Memory effects.* No contamination of the apparatus with  $C^{14}$  activity is ever observed, but a watch should be kept for possible contamination with tritium. This can occur in either of two places: in the combustion tube or in the apparatus where tritio-butane is manipulated. As described in Chapter IX, contamination by butane, which is slightly soluble in vacuum grease, is observed only in stopcocks and taper joints which should therefore be cleaned and regreased whenever they have been in prolonged contact with butane of high specific activity. The mere passage of butane through stopcocks as it is transferred from one part of the apparatus to another does not cause contamination. It should be noted that blobs of grease sucked into the line from over-greased stopcocks can be very troublesome and should be most carefully avoided. If grease does inadvertantly get into the apparatus and cannot be reached with forceps and tissue paper, then it can often be burnt away by passing a stream of oxygen through the line and heating the contaminated region with a semiluminous flame.

Contamination of the combustion tube is not normally observed but it should be monitored after the combustion of an unusually active sample. Monitoring is most conveniently done by allowing a counter full of inactive carbon dioxide at 20 cm. mercury pressure to expand into the evacuated furnace. The counter is then charged with the usual 2 cm. mercury pressure of carbon disulphide and the carbon dioxide condensed back into it. Tritium activity in the combustion tube will usually follow the carbon dioxide and give it an increased counting rate. Monitoring can be carried out more reliably by combusting a sample of inactive glucose and assaying the products by the usual methods. If contamination is found, further flushing of the hot tube with oxygen is necessary. Little trouble of this sort will normally be encountered, however, if the combustion tube is kept flushed with

oxygen between runs and tritium activities are kept as low as conveniently possible. It is best if samples to be combusted contain a total of not more than  $10^5$  counts/min. and preferably less than  $10^4$  counts/min. This can usually be arranged in advance by adjusting the dose of labeled material used in the experiment. The assay of high activities, when necessary, can be accomplished by wet combustion as described in Chapter IX. In this method, the sample is oxidized with Van Slyke combustion fluid which is then suitably diluted and 10 mg. converted to butane and counted. The procedure is very rapid and provides a means of avoiding high activities in the combustion train.

*Reproducibility of results.* The method has been tested by the assay in triplicate of samples containing  $C^{13}$ ,  $C^{14}$ , and tritium (1). These samples were the calcium salts of mixed total fatty acids extracted from mammary gland slices which had been incubated in a medium containing glucose, uniformly labeled with  $C^{14}$ , and sodium acetate labeled in the methyl group with tritium and in the carboxyl group with  $C^{13}$ .

Three samples weighing 8.8 mg., 11.1 mg., and 13.1 mg., respectively, were combusted, and the yields of carbon dioxide per unit weight of material found to agree within 1%. The three carbon dioxide samples were each divided into three portions. Two were counted and one was assayed mass spectrometrically for  $C^{13}$ . The three water samples were converted into butane and each butane sample divided into two and counted separately. The six values thus obtained for the specific activity of the carbon were spread over a range of 3.7%, which is better than  $\pm 2\%$  of the mean. The abundance of  $C^{13}$  (atoms per cent excess) and the specific activities of the six tritio-butane samples were also within  $\pm 2\%$  of the mean values.

#### REFERENCES

1. R. F. GLASCOCK, *Biochem. J.* **52**, 699, (1952).
2. W. G. DUNCOMBE and R. F. GLASCOCK, unpublished.
3. The Thermal Syndicate Ltd., 12-14 Old Pye Street, London, S.W.1; and 14 Bixley Heath, Lynbrook, New York.
4. J. E. S. BRADLEY, R. C. HOLLOWAY, and A. S. MCFARLANE, private communication; and *Biochem. J.* **57**, 192 (1954).

## CHAPTER VII

### THE DETERMINATION OF DEUTERIUM

Both isotopes of hydrogen present special problems in assay of which the most important are those arising from exchange reactions. Exchangeable hydrogen is ubiquitous: it is found as a tenaciously adsorbed layer of water molecules even on the inner walls of instruments which are operated under high vacuum, it is present as OH groups of the silicates of glass, and it is present in other exchangeable forms in metals. Any procedure, therefore, which produces excited hydrogen ions is likely to bring about exchange. This is most likely to occur just where it is least wanted either as a result of the electron bombardment of water vapor or hydrogen gas inside a mass spectrometer or under the influence of the discharge inside a counter. Thus the apparent isotope content of one sample will be affected by that of the sample previously analyzed, giving rise to the so-called memory effects.

If analyzed in the form of hydrogen gas, deuterium suffers from the further disadvantage that its determination in most commercial mass spectrometers is complicated by the unique properties of hydrogen and deuterium—in particular by their large relative mass difference. One possible consequence of this mass difference is isotopic fractionation within the spectrometer because the two different kinds of molecules  $H_2$  and  $HD$  have different diffusion and pumping rates. Another possible consequence is that the two kinds of ions  $H_2^+$  and  $HD^+$  may be appreciably but differently affected by the magnetic field controlling the electron beam. A still further difficulty is the formation of  $H_3^+$  ions which have the same mass as  $HD^+$  ions but whose contribution to the mass 3 peak varies with the square of the pressure within the instrument. The mass spectrometric analysis of deuterium-hydrogen mixtures in commercial instruments is not



therefore entirely straightforward. Calibration against standard deuterium-hydrogen samples is necessary to correct for the various errors mentioned above and considerable experience is essential if reliable results are to be obtained.

The use of water vapor in the mass spectrometer presents even greater difficulties than does hydrogen; this is not only because it exchanges much more readily with adsorbed and combined hydrogen in the walls of the instrument, but also because of the multiplicity of ions formed under electron bombardment. According to Washburn, Berry, and Hall (1) the mass peak 19 is contributed to not only by  $\text{HO}^{16}\text{D}^+$  but also by  $\text{HO}^{17}\text{H}^+$ ,  $\text{O}^{17}\text{D}^+$ ,  $\text{O}^{18}\text{H}^+$ , and  $\text{H}_3\text{O}^{16+}$ . Of these, the last,  $\text{H}_3\text{O}^{16+}$ , is the most troublesome because the magnitude of its contribution varies with the instrument, the voltage applied to the electrodes of the ion source, and the pressure of the water vapor. It is therefore of interest to learn (1953) that by special modifications of commercial mass spectrometers these workers have succeeded in reducing the formation of these ions and in reducing exchange reactions so that they are able to measure the deuterium content of small water samples with an accuracy of  $\pm 0.002$  atom per cent. The modifications necessary are briefly described in the original paper, and it is obvious that if instruments embodying them become commercially available they will be of great benefit to many workers. Meanwhile, although the mass spectrometric determination of deuterium will, in time, doubtlessly supersede density methods, the cheapness and simplicity of the latter still make them attractive. A brief description of the most accurate of them is therefore included.

### Determination of Deuterium by the Falling Drop Method

The determination of the density of a drop of water by measuring its rate of fall through an immiscible liquid was first devised by Barbour and Hamilton (2) in 1924 and was subsequently applied by others, notably Keston, Rittenberg, and Schoenheimer (3), to the determination of deuterium oxide in water. These workers used *o*-fluorotoluene, whose density at the convenient temperature of  $27^\circ$  is such that a water drop 7–10  $\mu\text{l}$ . in volume



and containing 0–3% deuterium oxide falls at a convenient rate (15 cm. in 25–180 sec.). The deuterium concentration is deduced from the time of fall by means of a calibration graph. Keston, Rittenberg, and Schoenheimer plotted deuterium concentration against the reciprocal of the time of fall over the standard distance. A greater degree of accuracy is achieved by plotting deuterium content against  $\frac{1}{T} - \frac{1}{T_0}$  where  $T_0$  is the time of fall of pure water and  $T$  the time of fall of the deuterium oxide-containing samples (see Fig. 52). This compensates for slight variations in temperature of the water bath since  $1/T$  and  $1/T_0$  will be affected approximately equally. For a discussion of the underlying theory and a description of methods believed to give even greater accuracy, the paper by Cohn (4) should be consulted. It has been found in the present author's laboratory, however, that the errors arising from the translation of dropping time into deuterium content are negligible compared with those arising in other parts of most experiments.

For the determination of deuterium in an organic compound, the sample must first be combusted and the water purified. Since 100 mg. water are required for the convenient application of the falling drop method, the combustion must be on a larger scale than most of those discussed in Chapters V and VI. The resultant water must be submitted to a rigorous purification procedure. The original technique of Keston, Rittenberg, and Schoenheimer, which has undergone but little modification by later workers, is quite satisfactory and is standard in many laboratories.

#### *Method of Keston, Rittenberg, and Schoenheimer*

*Combustion.* These authors use a simple combustion train (Fig. 47) in which oxygen is passed through concentrated sulphuric acid in *g* and then through a concentric trap *h* cooled in a solid carbon dioxide bath before entering a quartz combustion tube *c*. This tube is 32 in. long and 1 in. in diameter and is packed over a distance of 18 in. with copper oxide. It is maintained at 750° by means of an electric furnace, auxiliary heating coils, *e* and *f*, being wound directly on the tube over the ends of the

packing to compensate for heat losses and thus maintain an even temperature. The combustion water is collected in a U-trap, *a*, cooled in solid carbon dioxide, which is attached to the end of the quartz tube by means of a standard taper joint. To prevent condensation in this joint, a strip of copper foil *k* wound around it is gently heated with a microburner during the course of the combustion.

*Water purification.* The additional apparatus shown in Fig. 48 is used. The various traps contain chemicals suitable for removing

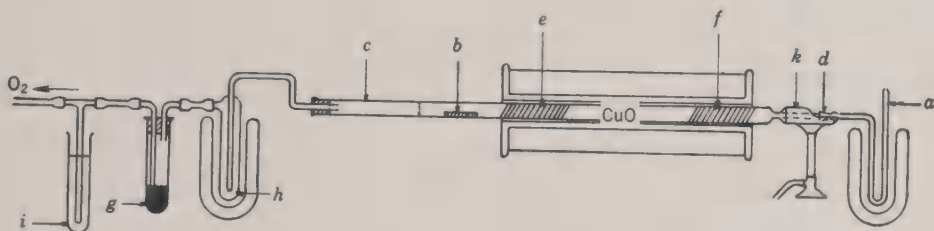


FIG. 47. Combustion train for deuterium compounds (Keston, Rittenberg, and Schoenheimer).

*a*: Chilled trap for collection of combustion water.

*b*: Combustion boat.

*c*: Quartz combustion tube.

*d*: Standard taper joint.

*e* and *f*: Auxiliary heating coils.

*g*: Sulphuric acid.

*h*: Concentric trap cooled in solid CO<sub>2</sub>.

*i*: Safety valve.

*k*: Copper collar.

all impurities likely to be present in the combustion water. These may be free halogens and nitric acid from halogens and nitrogen in the original compounds together with traces of volatile compounds which have been produced in the combustion train but have escaped complete oxidation. If halogens are present, a few centimeters of bright copper wire are added to the combustion water in *a* and left in contact with it for at least 12 hours. At the end of that time traces of nitric acid are removed by adding an excess of dry barium carbonate. The trap *a* is then connected to the distillation train, the water in it frozen by means of a solid carbon dioxide bath, and the line evacuated. After warming up, the water is distilled into trap *b* which contains a few milligrams

of dry chromic oxide ( $\text{CrO}_3$ ). Air is admitted through the magnesium perchlorate tube *h*, and the water is boiled gently. This completes the oxidation of organic substances and expels carbon dioxide. The water is next transferred *in vacuo* to trap *c* which contains potassium permanganate and potassium hydroxide or, better, calcium oxide since it has no exchangeable hydrogen. The water is then doubly redistilled first into the empty tube *d* and

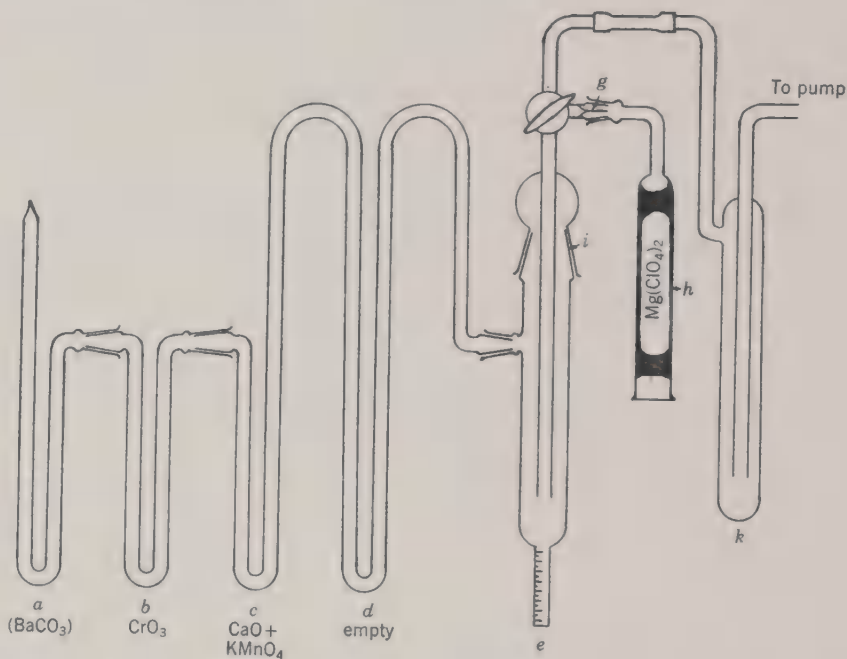


FIG. 48. Water distillation apparatus (Keston, Rittenberg, and Schoenheimer).

finally into the tube *e*, which is graduated in 0.05-ml. divisions. This trap is removed, stoppered, and the water taken for deuterium determination.

#### *Method Using Permanent Vacuum-line Technique*

In the present author's laboratory the method of Keston, Rittenberg, and Schoenheimer has been modified for use with the permanent vacuum-line apparatus already described (Figs. 1, 4, and 43). The apparatus shown in Fig. 43 could be used with but little alteration if the combustion tube and furnace *F*

were replaced by one large enough to deal with 150-mg. samples. It has been found better, however, to build another apparatus which is supported on the opposite side of the scaffolding (see Fig. 4). As this can be used for the combustion of both large and small samples, it can be used also for the assay of compounds containing  $C^{13}$ ,  $C^{14}$ , and tritium. This duplication of the apparatus more than doubles the number of analyses that can be performed in a day.

This second apparatus, used both for the combustion of deuterio-compounds and the purification of the combustion water, is shown in Fig. 49. It includes a manometric chamber for the measurement of carbon dioxide and auxiliary apparatus for the filling of counters by the methods already described (page 83) in case the sample also contains labeled carbon.

The diagram is largely self-explanatory, and the apparatus is very similar to that shown in Fig. 43. It differs chiefly in incorporating a larger combustion tube and furnace ( $F$ ). The combustion tube is 75 cm. long and 3 cm. in diameter, about 35 cm. of its length being packed with copper oxide which is maintained at  $750^\circ$  with an electric furnace. The combustion of 150-mg. samples of organic compounds proceeds smoothly and quantitatively if weighed into a porcelain boat, covered with freshly ignited copper oxide powder, and then heated very gently in a stream of oxygen. As in the apparatus previously described, the oxygen is drawn through the apparatus by means of the pumps, its rate of flow being controlled with a control stopcock ( $S_{38}$ ). Water and carbon dioxide are stripped from the gas stream by cooling the traps  $T_8$  and  $T_9$  in liquid air. The optimum flow rates, as determined by combusting standard samples of pure compounds, is 100 ml./min. in the author's apparatus. After the initial decomposition of the compound, the combustion is completed by heating the boat strongly with a Meker burner for 5 min. The contents of the traps are combined, the carbon dioxide is sublimed away from the water at a temperature of  $-78^\circ$ , and the water then sublimed *in vacuo* into the tube beneath stopcock  $S_{41}$ , which contains a few milligrams of fresh chromic oxide ( $CrO_3$ ).



The technique differs here from that used by Keston, Rittenberg, and Schoenheimer in that air is not admitted to the apparatus every time the water is reacted with a chemical. It is merely boiled under its own vapor pressure. In the presence of

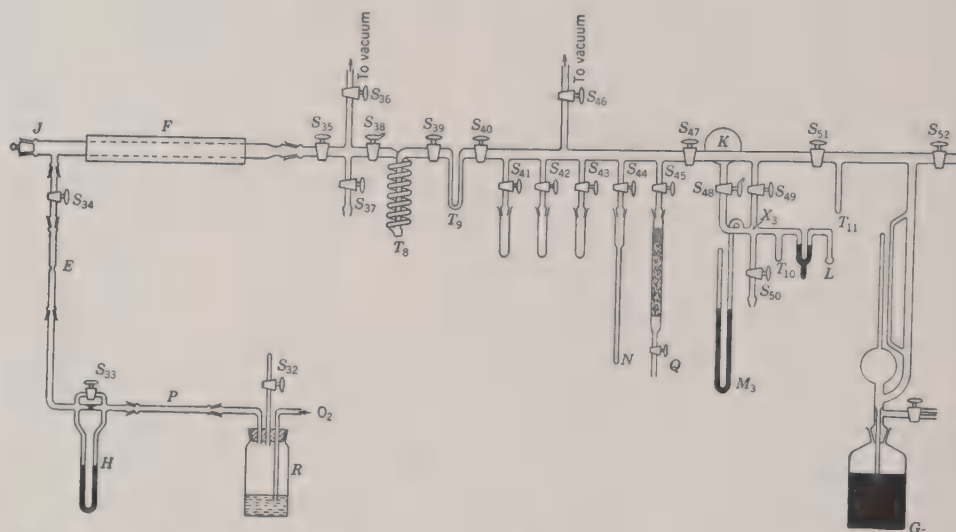


FIG. 49. Vacuum-line combustion train for deuterium compounds. This is similar to and partly duplicates the apparatus shown in Fig. 43. The combustion tube and furnace *F* is larger than that shown in Figs. 42 and 43 so that 100-mg. samples can be burned. If the sample contains  $C^{14}$  as well as deuterium, the  $C^{14}O_2$  is manipulated in the manometric space  $X_3$  and counted in a counter attached to the joint below stopcock  $S_{50}$ .

*E*: Oxygen purification tube.

$G_3$ : McLeod gauge.

*H*: Flowmeter.

*J*: Joint and stopper (B29).

*K*:  $CO_2$  reservoir.

*L*:  $CS_2$  reservoir.

$M_3$ : Manometer.

*P*: Platinized asbestos tube.

*R*: Bubbler and oxygen reservoir.

$S_{32}$ – $S_{52}$ : Stopcocks.

$T_8$ – $T_{11}$ : Traps and condensation tubes.

*N*: Tube for collection of purified combustion water.

*Q*: Air inlet and purifier.

$X_3$ : Manometric space.

air it is difficult to condense down all the water vapor that enters the gas phase during the boiling, but this is easily achieved in its absence. After boiling with chromic oxide, the tube is immersed in a solid carbon dioxide bath to freeze down the water, and the tube is exposed to the main pumps for a few seconds to

pump away any carbon dioxide formed. The water is then sublimed into the next tube ( $S_{42}$ ), which contains barium carbonate. It is boiled, frozen down, and exposed to the pumps. If halogen is suspected as a contaminant, an extra tube containing bright copper wire must be employed before the other treatments. Finally the water is twice re-sublimed, first into the tube beneath  $S_{43}$  and then into the bottom of the narrow tube  $N$  beneath  $S_{44}$ . This tube has been previously cleaned by boiling with fresh chromic acid cleaning fluid, washing, and drying. Once the water has been condensed in it, dry, carbon dioxide-free air is admitted through the purification tube  $Q$  beneath stopcock  $S_{45}$  and the tube sealed off a few centimeters above the water sample and removed. The normal water sample, which is dropped at the same time as the unknown, is introduced into the apparatus through stopcock  $S_{37}$  and subjected to the same purification treatment. Both samples are then taken to the falling drop apparatus.

### *Falling Drop Apparatus*

The apparatus described here is modeled largely on that described by Popják (5), with a few small modifications. It is in routine use in the author's laboratory and has been found to give satisfactory results.

*Water bath.* This consists of a glass tank of 40–50 liters capacity. The wall should be optically clear so that the falling water drop in the tube of *o*-fluorotoluene can be closely and accurately observed. If such a tank is not available it can be constructed from sheet metal with glass or transparent plastic (Perspex) panels back and front. The bath is 50 cm. deep and 35 cm. square. It is housed in a wooden box large enough to allow about 3 cm. of lagging all around with the exception of a 10-cm. panel front and back to permit observation and illumination. It is kept in a room maintained at  $20^{\circ} \pm 2^{\circ}$ . If larger fluctuations in the ambient temperature are permitted then the ultimate control of the temperature of the bath to within  $0.001^{\circ}$  will not be possible.

The water must be well stirred without excessive vibration. Popják describes a centrifugal stirrer which rotates fast enough

to take in water at the bottom of a tube and to eject it at the top. It has been found, however, that a simple stirrer consisting of several vanes on a rotating shaft gives adequate stirring. The stirrer must be independently supported so that no vibrations are transmitted to the tank and dropping tube.

*Thermostat.* This is a toluene-mercury thermoregulator of the conventional type but with a toluene reservoir in the form of

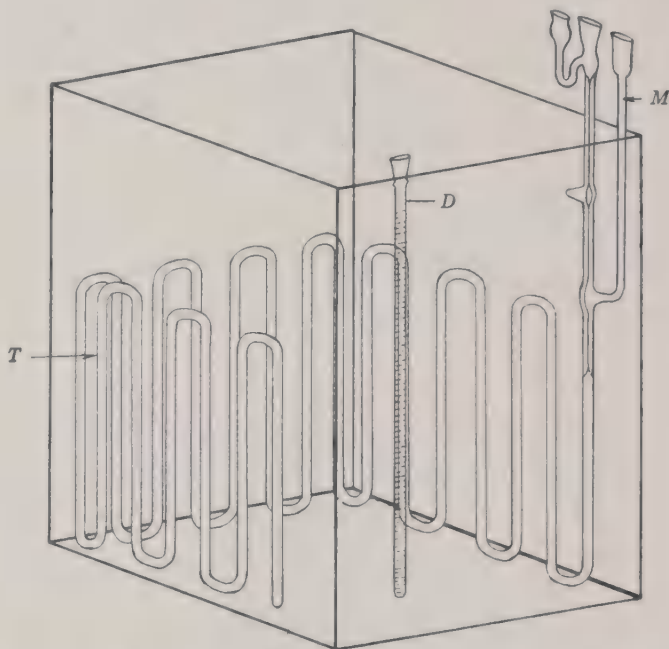


FIG. 50. Falling drop apparatus (in part), showing tank, toluene bulb-thermoregulator (*T*), proportioning head (*M*), and tube of *o*-fluorotoluene (*D*).

a series of U-bends 30 cm. high and about 5 cm. apart around three sides of the bath (see Fig. 50). It is made from 5 meters of 12-mm. tubing and contains about 600 ml. toluene. The thermoregulator is operated with the usual apparatus consisting of coarse and fine mercury adjustment, electronic relay (Sunvic), and proportioning head.

*The heater.* A bare nichrome wire is wound on a length of glass tubing bent into the form of a 25-cm. square which is held clear of the metal bottom of the tank with glass feet. The wire has

a resistance of 3.5 ohms and is energized with a 12-volt supply from a transformer. The thermoregulator relay controls the main input to the transformer since the relay is designed to operate only at line voltage.

*The o-fluorotoluene.* This is contained in a tube about 55 cm. long and 15 mm. wide, provided with a glass stopper. Two marks are engraved on the tube 15 cm. apart, the upper one being 20 cm. below the surface of the liquid. This allows the drop to come into equilibrium and to attain a constant rate of fall before timing commences. The tube is clamped in the water bath about 10 cm. behind the observation panel.

*Setting up.* After setting up the thermoregulator in the usual way and getting it adjusted to  $27.2^{\circ}$ , the accuracy of temperature control is checked by means of heating and cooling curves. With the heater switched permanently on, a heating curve is plotted over a range of  $0.1^{\circ}$  using a Beckmann thermometer. A cooling curve is also plotted over about the same range with the heater off. From these two plots the mean rate of heating and cooling can be read. In a particular case both were found to be 0.00011 degrees/sec. When this has been done, the thermoregulator is switched into service and the *on* and *off* times measured by observing the pilot light. In the particular case mentioned, the *on* time was 16 sec. and the *off* time 17 sec. This indicates a temperature fluctuation of  $\pm 0.0009^{\circ}$  C.

*Micropipette.* The micropipette is designed to deliver accurately measured drops about  $10\ \mu\text{l.}$  in volume. Keston, Rittenberg, and Schoenheimer (3) have described the pattern used by them, but in the present author's laboratory that described by Popják (5) is used (Fig. 51). It is constructed from stainless steel, with a micrometer-operated plunger by means of which a column of mercury is moved in a glass capillary tube. This tube must be carefully made, the end being beveled as shown in the figure. This can be achieved with a device made by drilling a concavity in the end of a length of  $\frac{1}{4}$ -in. brass rod, filling it with a paste of 700-mesh carborundum and water, and grinding by hand until a uniformly beveled edge is achieved. Popják himself uses a dentist's drill. This beveling is necessary to promote the



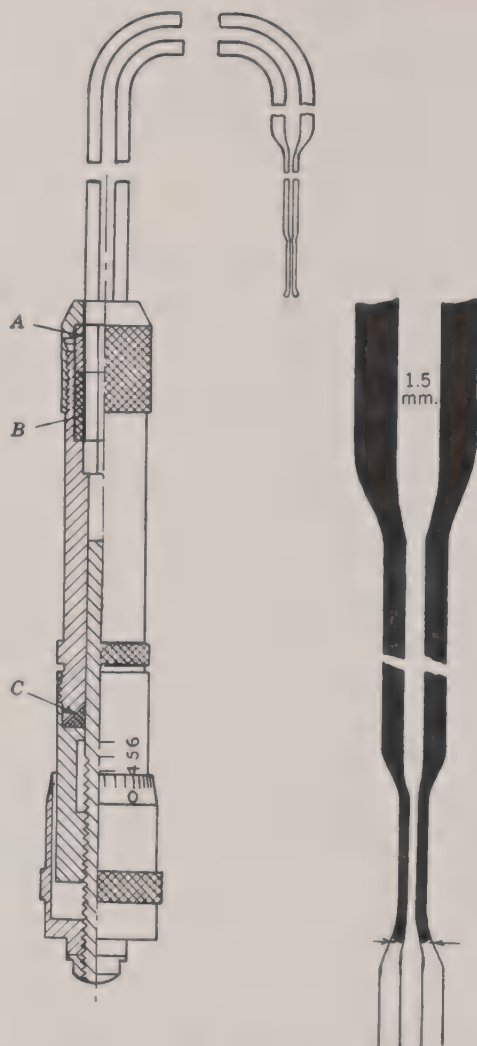


FIG. 51. Micropipette (Popják). A: metal ring which compresses rubber sleeve; B: rubber sleeve; C: neoprene gland washer to provide air-tight seal between piston and barrel. Other parts are self-explanatory.

ready detachment of drops. "Creeping" of the water up the outside is prevented by treatment with silicone (Dow Corning silicone fluid DC 1107).

The micropipette is supported in a rack and pinion by means of which the glass tube may be lowered, so that its tip dips below the *o*-fluorotoluene, or swung clear for charging with water.

*Dropping.* By manipulating the micrometer, the mercury is brought to the tip of the glass tube which is then immersed in the sample of water to be dropped. If the pipette has been used before and is wet with the previous sample it must be washed out with about 5  $\mu$ l. of the new sample. About 15  $\mu$ l. are then

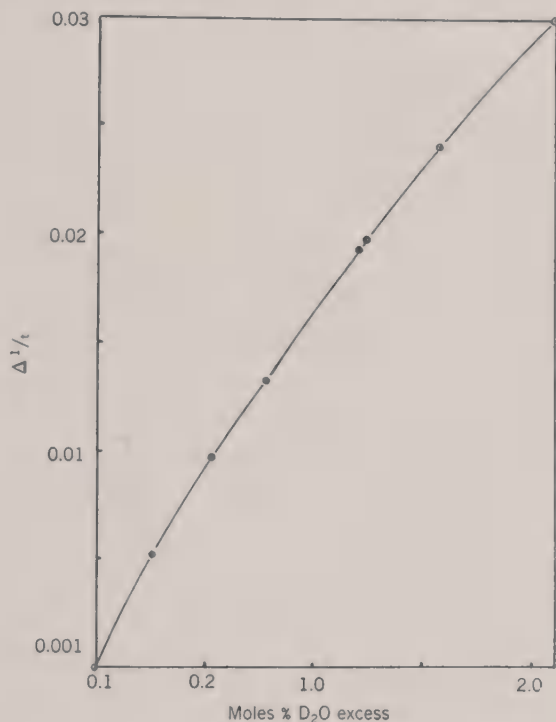


FIG. 52. Calibration curve. Plot of  $\Delta \frac{1}{T}$  against deuterium content of water samples.  $\Delta \frac{1}{T} = \frac{1}{T} - \frac{1}{T_0}$  where  $T$  = dropping time of sample and  $T_0$  = dropping time of pure water.

drawn into it and the tip of the tube dipped about 5 mm. below the surface of the *o*-fluorotoluene. A standard volume is then expelled by means of the micrometer, giving rise to a small spherical drop which hangs from the tip of the tube. The tube is then gently withdrawn from the fluid, the drop becoming detached at the surface. Several such drops, all of the same size, are timed over the measured distance using a stopwatch measuring

to 0.01 sec. The range of times for a given sample should not exceed 1% corresponding to an absolute error of 0.02 atoms per cent excess deuterium. The mean dropping time  $T_0$  of normal water which has been through the purification process is measured immediately after that of each sample of combustion water. Then, from the mean dropping time of each sample, the value of  $\frac{1}{T} - \frac{1}{T_0}$  is calculated and the corresponding deuterium content found by reference to a calibration graph (see Fig. 52) which has been previously prepared from a series of carefully weighed dilutions of pure deuterium oxide.

### The Mass Spectrometric Determination of Deuterium, Using Hydrogen

In many experiments it is difficult or impossible to obtain the 100 mg. of combustion water which is required for the application of density methods. For this and other reasons, mass spectrometric methods which are applicable to much smaller samples are preferred by many workers. As stated earlier, the analysis of water in the mass spectrometer presents many difficulties and it is usual to convert the water into some other gas with fewer undesirable properties. Hydrogen itself is the gas most usually chosen and many methods for the quantitative reduction of water have been described. Most of them are very similar and involve the reaction of steam with a reducing metal at elevated temperatures. Thus Allen and Ruben (6) carry out the reduction by treating steam with magnesium turnings at 620° whereas Henriques and Margnetti (7) use magnesium amalgam at 400°. Biggs, Kritchevsky, and Kirk (8) use lithium aluminum hydride dissolved in anhydrous Carbitol, and Smith and Marshall (9) use calcium metal. These methods are all designed for the 100–200-mg. scale, although, no doubt, some of them could be scaled down successfully. The method of Graff and Rittenberg (10), and a modification of it by Dubbs (11), being designed for the 5–10-mg. scale appears to be the most suitable published method for achieving the reduction. It should be noted that since hydrogen can also be used in a counter or ionization chamber, most of the

methods described for the production of deuterium-hydrogen are equally applicable to the preparation of tritium-hydrogen and *vice versa*.

## THE REDUCTION OF WATER TO HYDROGEN

### *Method of Graff and Rittenberg*

These authors (10) use a composite apparatus designed for the combustion on the 5-mg. scale of organic samples and the reduction of combustion water, in a separate operation, with hot zinc. The apparatus is shown in Fig. 53.

*The combustion tube.* This tube, *A*, consists of a quartz or Vicor tube, 10 mm. wide, packed with copper oxide or platinum wool kept in place with silver gauze. It is maintained at  $750^{\circ}$  by means of an electric furnace. Oxygen is passed through the tube at atmospheric pressure at the rate of three bubbles a second and is dried on the way by means of a tube filled with magnesium perchlorate. The combustion tube is connected to the rest of the apparatus with a short length of thick rubber tubing.

*The converter tube.* This tube, *B*, is made from 10-mm.-diameter glass fitted as a sleeve to the narrower outlet tube. The whole device is filled with 10-mesh zinc granules and kept at a temperature between  $384^{\circ}$  and  $415^{\circ}$ , depending on the age of the filling. The operating temperature gets progressively higher during the course of its useful life, which is about 200 samples.

*Procedure.* The sample is weighed into a platinum or porcelain boat and combusted as rapidly as possible "without exploding or burning in the boat." Stopcocks 1 and 2 are set so that the mixed gases from the combustion tube pass through the U-trap *C*, which is cooled in a solid carbon dioxide bath, and out through stopcock 5. No data are included in the published account of the method to prove that a quantitative yield of water is collected in trap *C*, but presumably it has been tested by the authors.

After the combustion is complete, stopcock 5 is closed, the oxygen supply tube is closed with a screw clamp, and the apparatus is evacuated with a mechanical pump connected to stopcock 6. As traces of air do not interfere with the mass spectro-



metric determination of hydrogen, a diffusion pump is not required. If the sample to be assayed is not an organic compound but the water of a solution such as blood or urine, it is distilled from a tube attached to the side arm *G* directly into trap *C*, care being taken to prevent the contamination of such small samples by atmospheric moisture.

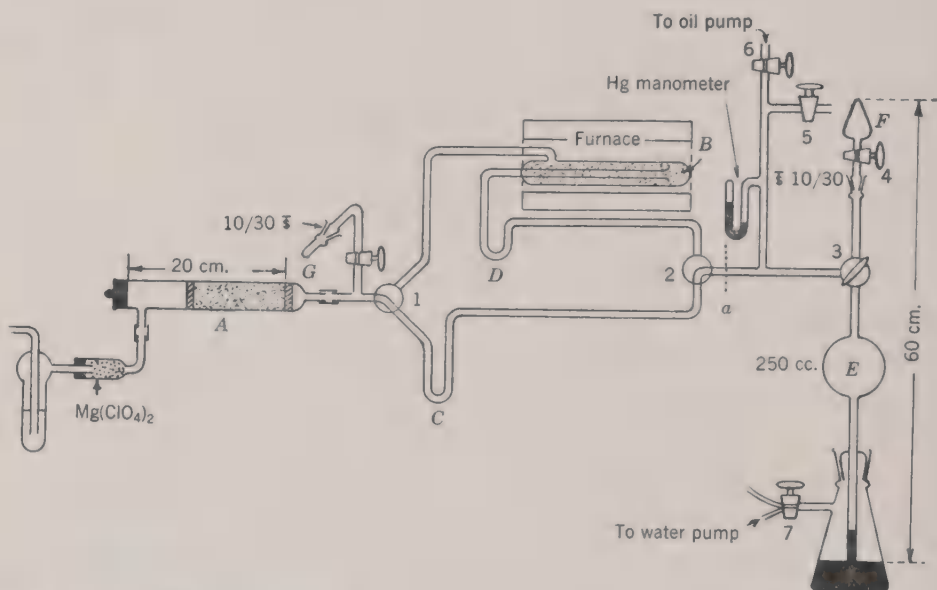


FIG. 53. Combustion apparatus and water reducer (Graff and Rittenberg).

A: Quartz or Vycor combustion tube at  $750^{\circ}$ .  
 B: Reducer, filled with zinc granules and kept at  $400^{\circ}$ .  
 C, D: U-traps.  
 E: Toepler pump.

F: Gas sample bulb.  
 G: Tube for the introduction of water samples into apparatus.  
 a: Point at which apparatus is cut for dismantling.  
 1-7: Stopcocks.

After the pressure has fallen below 5 mm., the combustion tube is isolated by turning stopcock 1, which is then adjusted so that trap *C* is connected to the converter tube *B*. The whole system is evacuated as far as possible, the vacuum being checked by using the Toepler pump *E* as a McLeod gauge, and stopcock 2 is adjusted so that the outlet from the converter tube communicates through trap *D* and stopcock 3 with the Toepler pump.

The solid carbon dioxide bath is removed from trap *C* and placed around *D* where it serves to trap any water that may escape reduction. As *C* warms to room temperature, the combustion water in it vaporizes into the converter tube where it is reduced to hydrogen, the process being indicated by a rising pressure on the manometer. This hydrogen is transferred to a bulb *F* attached to the joint on top of the Toepler pump. If the yield is not quantitative and unreacted water remains in trap *D*, it can be transferred back to trap *C* for re-cycling by changing over the bath of solid carbon dioxide and manipulating the appropriate stopcocks. By achieving quantitative conversion, isotopic fractionation is avoided.

When all the hydrogen is in the sample bulb *F*, stopcock 4 is closed, the mercury in the Toepler pump is brought to its original level, and the apparatus re-evacuated to be ready for the next determination.

*Memory effects.* It was found that when a large sample of deuterium oxide containing 86 atoms per cent excess deuterium was passed through the system, followed by a series of 5-mg. samples of normal palmitic acid, the hydrogen from them was contaminated with deuterium. This contamination was 1% in the first sample and diminished with each successive sample to 0.034% in the fourth. This retention of deuterium in the apparatus could neither be localized in any particular part nor could it be prevented. It was shown, furthermore, that the deuterium-containing compound retained in the apparatus had an exceedingly low vapor pressure and could not be removed by heating contaminated glass under vacuum nearly to its softening point. It was presumably in the form of silicate OD groups.

The effect of this contamination is reduced to a minimum by using only 2- to 5-mg. samples of material for combustion and by assaying each sample in duplicate. The errors, however, are not excessively high and can be tolerated in most experimental work.

### *Method of Dubbs*

This author (11) has succeeded in greatly simplifying the reduction apparatus and much reducing contamination by using

a small reaction vessel which can be replaced for each new determination if desired. Because of the small size of the reaction vessel the amount of exchange taking place is also much reduced.

*Apparatus.* Dubbs' apparatus is shown in Fig. 54. The reaction vessel consists simply of a 5-mm.-bore tube, 15 cm. long,

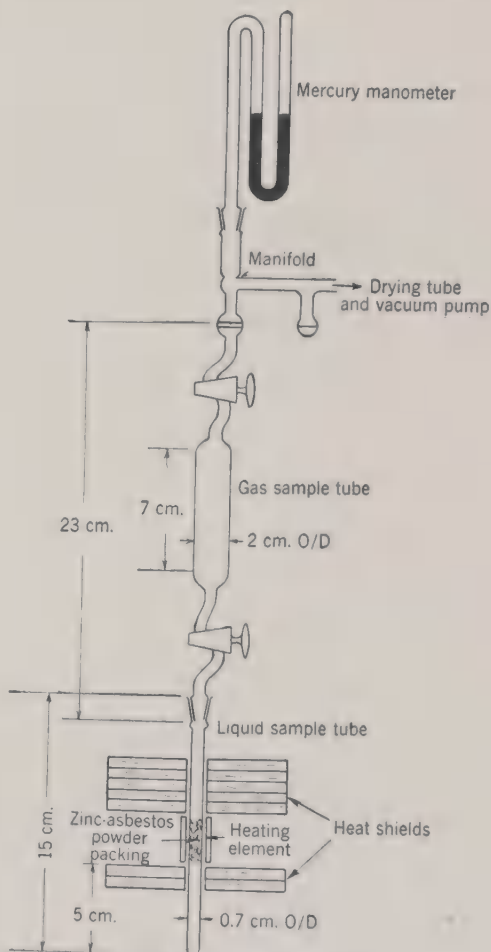


FIG. 54. Water reducer (Dubbs).

fitted with a standard taper joint. A 10-mg. sample of water is weighed into the bottom of it and frozen in a solid carbon dioxide bath. A ball of crumpled aluminum foil is then added, being supported by pips in the glass about 5 cm. from the bottom, and followed by a mixture of commercial zinc dust and asbestos powder to form a 3-cm. column. The reduction zone is provided

with an electric heater insulated from the rest of the apparatus with asbestos boards. The gas sample tube is attached to the reaction vessel by means of a standard taper joint. It consists of a cylindrical vessel of about 20 ml. capacity with a stopcock at each end which can, in its turn, be attached to a vacuum manifold through a spherical joint.

*Reduction.* With the lower part of the tube still cooled in the solid carbon dioxide bath, the apparatus is evacuated and the heater then run at  $400^\circ$  to remove water retained in the reduction mixture. Heating is continued until no further evolution of hydrogen is indicated by the manometer. After pumping away the hydrogen, the reduction of the sample is started by replacing the cold bath by a water bath at  $50^\circ$ . The reaction proceeds to completion in about 20 min. and the hydrogen collected in the gas sample tube is then transferred to the mass spectrometer for analysis.

TABLE 4

Sequence	$D_2O$ present (atom % excess)	$D_2O$ found			
		Previous apparatus		New apparatus	
		A	B	C	D
1	0.20	0.182	0.226	0.199	0.202
2	0.00	0.030	0.050	0.002	-0.005
3	0.00	0.007	—	0.003	0.002

The same reduction trains were used for the series *A* and *B*; the same gas sample tubes for the series *C* and *D*.

It will be noticed that the retention of deuterium in the apparatus is less than one-tenth of that observed with a Graff and Rittenberg reduction train.

Because the apparatus is so simple, many sets can be made and different groups reserved for the determination of deuterium in various ranges of concentration. Table 4, published by Dubbs, shows the memory effect of his apparatus compared with one set up and operated according to the instructions of Graff and Rittenberg ("previous apparatus").

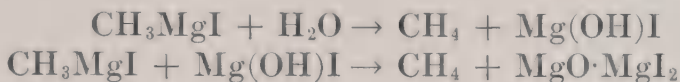


### The Mass Spectrometric Determination of Deuterium Using Hydrocarbons

Although hydrogen has fewer disadvantages than water as a medium for the mass spectrometric determination of deuterium it is still not as convenient as could be wished. The exchange reactions occurring both within the reducing apparatus and in the mass spectrometer itself cannot be entirely eliminated and there are, in addition, the difficulties, already summarized, arising from the relatively big difference in mass between  $H_2$  and  $HD$  and from the formation of spurious, mass 3 ions ( $H_3^+$ ). Some workers have recommended the use of hydrocarbons because they show none of these properties. The hydrogen, being stably bound, undergoes no exchange reactions with substances in the walls of the apparatus, and the relative mass difference between normal and deuterium-containing molecules is so small that no differential pumping or diffusion occurs. There are several reactions by means of which the hydrogen of water may be incorporated into a hydrocarbon, and it is therefore fairly easy to devise a method for the preparation of one which yields peaks in a convenient part of the mass spectrum. The mass spectrometric determination of deuterium in a hydrocarbon, however, suffers from the disadvantage that the mass peak due to the deuterium-containing ion is obscured by the mass peak of the corresponding  $C^{13}$ -containing ion i.e.,  $C^{12}DR$  has the same mass as  $C^{13}HR$ . The natural abundance of  $C^{13}$  is 1.1 atom per cent, and the mass spectrometric determination of deuterium using hydrocarbons is therefore apt to lose in accuracy what it gains in convenience. If a method of producing the hydrocarbon from reagents with a much reduced  $C^{13}$  content could be devised it would be very useful, but this does not seem feasible at present.

Of the methods available for the preparation of hydrocarbons from water all of those described in Chapters VIII and IX for the preparation of tritio-hydrocarbons could of course be applied to deuterium. The two gases which have been prepared for the specific purpose of deuterium assay are, however, *methane* and *ethane*. Orchin, Wender, and Friedel (12) prepare methane by

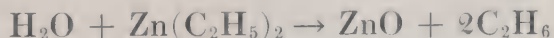
the action of water on methyl magnesium iodide, but have not been able to get the two mols of methane predicted from the equations



However, as only 1 mol is obtained in the Zerewitinoff method as usually carried out for the determination of active hydrogen, such a finding is not, perhaps, very surprising. These workers do not report their yields but assume that no isotopic fractionation occurs. The analysis in duplicate of many samples of deuterium oxide-water mixtures which gave results in good agreement with the expected values appears to justify this assumption. However their method lies open to the criticism put forward by Friedman and Irsa that, since the relative size of the mass peaks 16 and 17 is sought, there is danger of interference by hydroxyl ions. *Ethane* on the other hand can be analyzed for deuterium by comparison of the intensities of the 30 and 31 mass peaks ( $\text{C}_2\text{H}_6$  and  $\text{C}_2\text{H}_5\text{D}$ ) which are not contributed to by any of the commonly occurring impurities.

#### *Method of Friedman and Irsa*

These authors use zinc diethyl instead of a Grignard reagent and not only obtain a quantitative yield, which obviates the danger of isotopic fractionation, but introduce both the hydrogen atoms of water into the ethane produced



*Procedure.* The apparatus used is shown in Fig. 55. Zinc diethyl is contained in the reservoir *B*, and the water sample, which weighs about 10 mg., is introduced into the vessel *A*. This has previously been very carefully dried, and the water is added by means of a micropipette which is first flushed out several times with some of the water being analyzed. The drop is then frozen, using liquid nitrogen and *A* is evacuated. From 0.5 to 1.0 ml. of zinc diethyl is then distilled into *A* from the reservoir, the stopcock above *A* is closed, and the contents allowed to warm up.

As this occurs, bubbles of ethane can be seen forming in the liquid. The authors point out that it is advisable to use a large excess of zinc diethyl to avoid local overheating which may bring about violent decomposition reactions. This hazard naturally increases with sample size. The excess zinc diethyl can be recovered almost quantitatively at the end of the reaction. The reaction goes to 75% completion without warming and is completed by refluxing four or five times for 5 to 10 sec. at a time with a small flame.

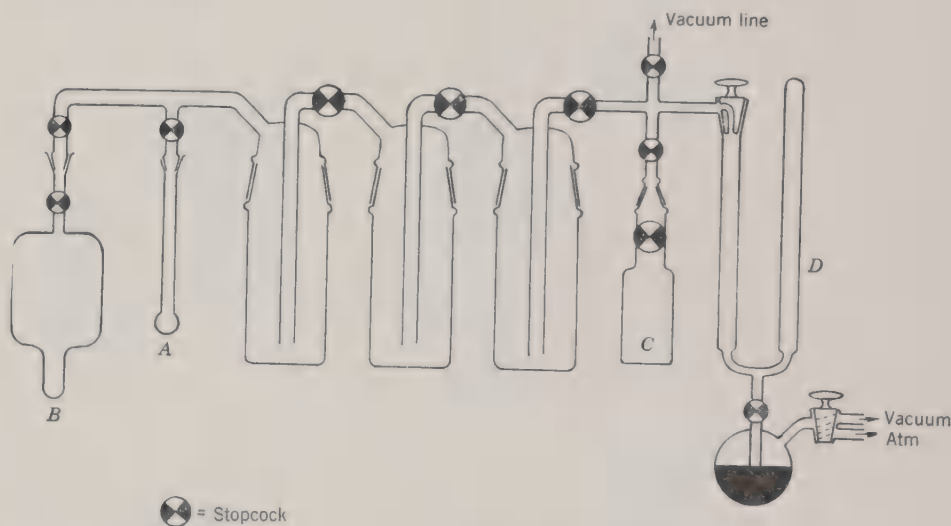


FIG. 55. Deuterio-ethane generator (Friedman and Irsa). A: reaction tube; B: zinc diethyl reservoir; C: gas sample bulb; D: Toepler pump.

The vessel A is cooled in liquid nitrogen to condense down all gas and vapor and the liquid nitrogen then replaced by a solid carbon dioxide-acetone bath. The ethane is condensed into the first trap by cooling it in liquid nitrogen, thus leaving behind any unreacted water vapor and the excess zinc diethyl. The stopcock above A is turned off, the contents again refluxed, and the extraction procedure repeated. The three traps are cooled with solid carbon dioxide in acetone, *sec.* butyl chloride-liquid nitrogen slush and liquid nitrogen, respectively. The ethane is purified by distilling through the first two traps into the third from which it is distilled at the temperature (not stated) of butyl-

chloride—liquid nitrogen into the sample bulb C. The Toepler pump shown in the diagram is used for measuring the ethane and compressing it into the reservoir bulb of the mass spectrometer.

No memory effects either in the reaction apparatus or in the spectrometer were observed by these workers. Thus, after running 99.8%  $\text{C}_2\text{H}_5\text{D}$ , pumping out for only 2 min., and then running normal ethane in the spectrometer, they recorded only 0.1% contamination. Unfortunately, as already stated, a lower limit of sensitivity is set by the naturally occurring  $\text{C}^{13}$ . This limit was found to be 0.5 to 1.0 atom per cent excess deuterium, below which reliable deuterium values could not be obtained. This is much too high for most biological purposes in which deuterium abundances smaller by at least a factor of 10 must be measured. Above 0.5 atoms per cent excess deuterium, however, the observed deuterium contents of a number of water standards were within 0.3% of the true values.

*Effect of incomplete reaction.* By stopping the reaction at various stages the effect of incompleteness of reaction was investigated. It was found that a marked isotopic fractionation occurred even when the reaction was as much as 95% complete. The authors therefore conclude that most errors in the method arise in the preparation of ethane rather than in the mass spectrometry. The actual figures obtained on assaying a water sample containing 4 atoms per cent excess deuterium are shown in Table 5. This result is in contrast with that of Orchin, Wender,

TABLE 5  
FRACTIONATION OF DEUTERIUM IN ETHANE SYNTHESIS

% reaction	%D determined	% error
6.3	1.68	58
23.8	1.76	56
46.0	2.02	49
54.2	2.15	46
95.0	3.57	11

Values obtained on assaying a water sample known to contain 4 atoms per cent excess deuterium. (Friedman and Irsa.)



and Friedel (12), who use the Grignard reagent for the preparation of deuterio-methane, and with the finding of the present author, who uses the corresponding reaction for the preparation of tritiobutane. In neither case is isotopic fractionation observed even when the reaction is much less than 95% complete. Robinson (14) also claims that no fractionation occurs when an excess of radioactive water is reacted with methyl magnesium iodide even though the yield of methane is only 43% calculated on the water (85% calculated on the Grignard reagent). It would therefore appear that the fractionation observed by Friedman and Irsa is peculiar to the reaction used.

## REFERENCES

1. H. W. WASHBURN, C. E. BERRY, and L. G. HALL, *Anal. Chem.* **25**, 130 (1953).
2. H. G. BARBOUR and W. F. HAMILTON, *Am. J. Physiol.* **69**, 654 (1924).
3. A. S. KESTON, D. RITTENBERG, and R. SCHOENHEIMER, *J. Biol. Chem.* **122**, 227 (1937).
4. MILDRED COHN, in "Preparation and Measurement of Isotopic Tracers" (D. W. Wilson, A. O. C. Nier, and S. P. Reimann, eds.) p. 51, J. W. Edwards, Ann Arbor, Michigan, 1946.
5. G. POPJÁK, *Biochem. J.* **46**, 558 (1950).
6. M. B. ALLEN and S. RUBEN, *J. Am. Chem. Soc.* **64**, 948 (1942).
7. F. C. HENRIQUES, JR. and C. MARGNETTI, *Ind. Eng. Chem. Anal. Ed.* **18**, 420 (1946).
8. M. W. BIGGS, D. KRITCHEVSKY, and M. R. KIRK, *Anal. Chem.* **24**, 223 (1952).
9. G. N. SMITH and R. O. MARSHALL, *Arch. Biochem.* **39**, 395 (1952).
10. J. GRAFF and D. RITTENBERG, *Anal. Chem.* **24**, 878 (1952).
11. C. A. DUBBS, *Anal. Chem.* **25**, 828 (1953).
12. M. ORCHIN, I. WENDER, and R. A. FRIEDEL, *Anal. Chem.* **21**, 1072 (1949).
13. L. FRIEDMAN and A. P. IRSA, *Anal. Chem.* **24**, 876 (1952).
14. C. V. ROBINSON, *Rev. Sci. Instr.* **22**, 353 (1951).

## CHAPTER VIII

### THE DETERMINATION OF TRITIUM

When used with proper precautions, tritium, the radioactive isotope of hydrogen, is potentially extremely useful not only as a label for hydrogen itself but also as an auxiliary label for carbon. It has the very convenient half-life of thirteen years which is long enough for the decay occurring during the progress of most experiments to be negligible and short enough for very high activities to be possible. Thus tritium can be obtained as hydrogen gas containing about 97%  $T_2$  at an activity of 2.7 curies/standard ml. This is about 1000 times greater than the specific activity of isotopically pure  $C^{14}O_2$  even if it were obtainable. Tritium, however, has not been extensively used chiefly because of the difficulties of assaying it. It is the weakest radioactive substance known, emitting beta-particles with a maximum energy of only 0.015 Mev. From this it is obvious that solid counting techniques, applicable to  $C^{14}$  assay only with considerable loss of efficiency, will be useless for the assay of tritium. Thus the infinite thickness value for  $C^{14}$  is about 20 mg./cm.<sup>2</sup> but only 0.8 mg./cm.<sup>2</sup> for tritium (1). Gas counting is therefore the method of assay almost invariably resorted to, although it is noteworthy that Eidinoff and Knoll (1) have described the counting of tritium with an efficiency of about 2.6% in a windowless, methane, flow-type proportional counter and that scintillation counting is also receiving increasing attention. By this method Hayes and Gould (2) achieve an efficiency of about 6.5%. At present, therefore, the efficiency of counting tritium by methods not depending on gas phase measurements are only just about as good as those achieved by the simple, end-window, solid counting of  $C^{14}$ . If much increased counting efficiencies are required, gas phase assay must be used. Gas counting is also essential if the sample contains both  $C^{14}$  and tritium since a tedious and probably inaccurate procedure

would be necessary for the separate determination of each in a solid sample. If the tritium is combined in an organic compound its assay must almost inevitably mean two separate processes: combustion to water and conversion of the water to a gaseous form suitable for radioactive measurements.

*Choice of medium.* It is unfortunate that tritium cannot be conveniently counted in the form of water vapor, but the problem of exchange is just as intractable for tritium as it is for deuterium. It is true that some workers have counted water vapor itself in a gas counter, but the difficulties involved make it doubtful whether any time or trouble is saved compared with methods depending on the conversion of water to some other chemical substance. Counters will tolerate only a very low partial pressure of water vapor (1 to 2 mm.), and the procedures for eliminating the contamination which always occurs are very tedious.

Most of the published methods depend on the use of hydrogen gas either in an ionization chamber or counting tube, but contamination of the instrument by exchange and the consequent elevation of the background is reported by several workers. Thus Arrol and Wilson (3) found that considerable contamination of graphite-cathode glass counters occurred after counting tritium-hydrogen in the Geiger region. Bernstein and Ballentine (4) observed that a counter which had been subjected to an hour's pumping on the high-vacuum line after counting a sample of tritium-hydrogen plus methane in the proportional region still retained 12% of the activity of the original sample. This contamination was almost certainly due to exchange of excited hydrogen atoms with adsorbed water and silicate OH groups in the walls of the counter. Verly and co-workers (5) also use a methane-hydrogen mixture in the proportional region and claim 1% reproducibility. They do not comment on the degree of contamination of the counter observed after counting this mixture although, in view of Bernstein and Ballentine's finding, it does not seem likely that they avoided it altogether.

The successful application of ion-chamber techniques to radioactive measurements on hydrogen are reported by several groups of workers. These techniques have the advantage that much

less contamination occurs in ionization chambers than in counters. For example, Henriques and Margnetti (6) found no more variation in background after using tritium than after using  $C^{14}O_2$ . It is also to be noted, however, that when an ionization chamber is filled with tritium the ion current produced is only about one-tenth of that produced by the same activity of  $C^{14}$ . This is because the average energy of tritium beta particles is about ten times less than those of  $C^{14}$ . As tritium is available in much higher activities than is  $C^{14}$ , this need not necessarily be a great disadvantage.

Contamination by exchange is best avoided by the use of hydrocarbon gases. Whereas the determination of deuterium by the mass spectrometric analysis of hydrocarbons is interfered with by the naturally occurring isotope  $C^{13}$ , no analogous interference occurs in the counting of tritium. The choice of gas to be used is therefore decided by a consideration of its counting properties and ease of preparation and manipulation.

If precise measurements of the specific activity of a gas are to be made, samples of known volume must be introduced into the counter or ionization chamber. This obviously means manipulation in a vacuum system. Quantitative transfer of gases is much facilitated if they are completely condensable at the temperature of liquid air. It is also necessary to avoid isotopic fractionation in the preparation of the gas, and, since the mass ratio of tritium to hydrogen is even greater than that of deuterium to hydrogen, the danger is correspondingly greater and must be guarded against. Isotopic fractionation is best avoided by driving the reaction used to completion.

The counting properties of the compound chosen are a most important consideration. Thus, whereas radioactive measurements on almost any gas could be made in an ionization chamber, not all gases are equally useful in proportional or Geiger counters. White, Campbell, and Payne (7) found that counters filled with methane prepared from the action of water on aluminum carbide showed no plateau in the Geiger region but could be operated in the proportional region. The present author (8), seeking a gas which, unlike methane, would be both condensable at liquid air



temperature and countable in the Geiger region, chose *n*-butane. This gas was found to have excellent counting properties and could be simply prepared without isotopic fractionation by the action of water on the dry Grignard reagent.

*Scale.* In making a choice of method to be used, important considerations are the scale for which it is designed and the amount of material available for assay. Thus in experiments such as the investigation of water turnover in the animal body, where the supply of water is virtually unlimited, a method designed for the 250–500-mg. scale may well be convenient, although not necessarily more so than one designed for the 10-mg. scale. In other experiments such as those concerned with the incorporation of labeled substrates into particular metabolites by tissue slices *in vitro*, the specimen to be assayed may well be no larger than 10–20 mg. in weight. The technique of assay would then have to be one designed for that scale or capable of being scaled down. As this is not usually possible without starting another research project on assay techniques, workers setting up a tritium assay apparatus for the first time would be well advised to consider whether it will be applicable to the smallest samples likely to be met with in their work.

The choice of instrument is also affected by sample size. Generally speaking, ionization chambers are designed to hold large volumes of gas and are useful where the sample is large but of low specific activity. Counters, on the other hand, usually hold less than 100 standard ml., and there is no advantage in preparing more gas than this; 10 ml. of gas is usually sufficient for accurate assay by counting techniques and it can usually be obtained by working on the 10-mg. scale.

### Water Vapor as a Medium for Tritium Assay

#### *Method of Pace, Kline, Schachman, and Harfenist*

These workers (9) use a Geiger counter containing alcohol vapor at a pressure of 2.5 cm. mixed with argon at a pressure of 2 cm. This mixture is unusual since the argon-alcohol ratio is usually about 10 to 1. Radioactive water vapor at a partial pres-

sure not exceeding 2 mm. mercury is added to this mixture for assay.

*Apparatus.* The apparatus is shown in Fig. 56. There are two McLeod gauges, one of low pressure range (*J*), and of a design similar to that already described (page 34), and one of a high

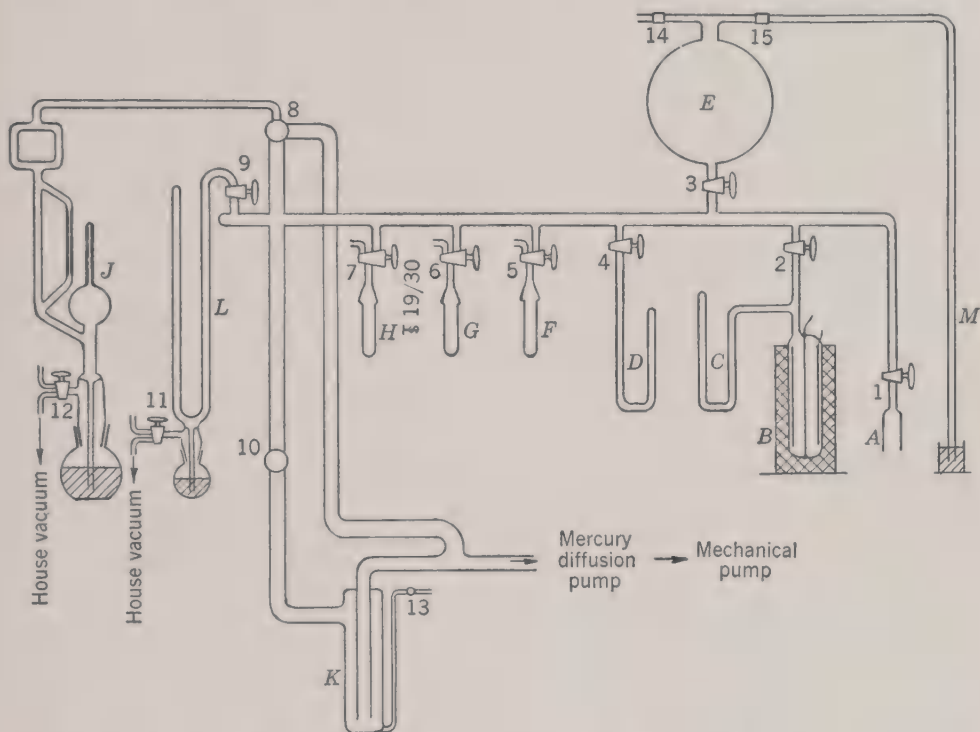


FIG. 56. Manifold for assay of radioactive water (Pace, Kline, Schachman, and Harfenist).

A: Calcium chloride tube.

B: Lead shielded counter.

C and D: Manometers.

E: Argon storage bulb.

F: Inactive-water storage tube.

G: Radioactive-water storage tube.

H: Ethyl alcohol storage tube.

J: Low-range McLeod gauge.

K: Liquid-air trap.

L: High-range McLeod gauge.

M: Manometer for argon bulb.

1-15: Stopcocks.

pressure range (*L*). The gauge *J* is for measuring low gas pressure and is used chiefly for checking the vacuum. The high-range gauge, *L*, which works on exactly the same principle as the other is used to measure water vapor pressures of 0.4 mm. to 2.2 mm.

In the side arms *H*, *G*, and *F* are stored ethyl alcohol, radio-

active water, and inactive water, respectively. Argon is stored in the reservoir *E*, the manometer *M* indicating the pressure available. *D* and *C* are manometers attached to the main manifold and to the counter, respectively.

The counter, which is permanently connected to the main vacuum apparatus, is unusually large so that it can contain a reasonable amount of water vapor at the low partial pressure used. Its internal volume is approximately 1 liter and, at the maximum permissible water vapor pressure of 2 mm., it will therefore hold just under 3 standard ml. It is made of glass with a copper screen cathode 5 cm. in diameter and 30 cm. long. The anode wire is 0.01-in. tungsten. The counter is protected with a lead shield 2 in. thick which reduces the background counting rate to about 200 counts/min., which is half the unscreened value.

*Procedure.* The manifold and counter are evacuated to a pressure of less than  $3 \times 10^{-4}$  mm. as shown by the McLeod gauge *J*. Radioactive water vapor from tube *G* is then admitted slowly to the manifold through stopcock 6, care being taken to ensure that the pressure registered on the manometer *D* does not rise above 2 mm. The pressure is then measured accurately by means of the high-range McLeod gauge *L*, stopcock 2 is closed to isolate the counter, and the manifold is evacuated.

Alcohol and argon must next be added. This is done by opening stopcock 7 to fill the manifold with alcohol vapor and then opening stopcock 2 until a pressure of 2.7 cm. is indicated on the manometer *C*. The excess alcohol in the manifold is then pumped away and the pressure raised to 4.7 cm. with argon from the reservoir using a similar procedure. The alcohol must be put in first because its vapor pressure is only about 4 cm. at room temperature and it would not be possible to raise the total pressure to 4.7 cm. if it were added last. The tube is permitted to stand about 10 min. before counting. It has an operating potential of 1400 volts and a plateau extending over 300 volts. The slope is not stated in the original publication. Pace and co-workers recommend that at least three measurements of 20,000 counts each should be made with each filling of the tube. The counting rate increases slowly during the first 20,000 counts and then becomes constant. The first reading is therefore disregarded and the mean

of the second two recorded. It was found that in 90% of determinations these two counts were within 1.1% of their mean. Background counts are measured in exactly the same manner after introducing inactive water from tube *F* into the counter.

*Contamination of counters.* When a counter, which had been used to measure activities of about 500 counts/min., was simply evacuated and refilled using inactive water vapor, the background counting rate did not return to normal. Decontamination was achieved by alternately evacuating and flushing eight times with inactive water vapor at a pressure of about 1.7 cm., and finally admitting air. The counter was then re-evacuated and filled. This procedure was used after each determination.

Since only one counter, which is a fixture in the apparatus, is used and therefore every count is made under exactly the same geometry, specific activities can be expressed as counting rate per unit water vapor pressure. It is recommended by the authors that at least four counts at different partial pressures should be made and the specific activity found by drawing the best straight line through a plot of activity against water vapor pressure and measuring its slope. They found that the mean standard error was only  $\pm 3.7\%$ .

The method described here could no doubt be improved by using detachable counters, thus making possible the use of several at a time, and by calibrating the high-range McLeod gauge in volume or weight units. The remaining disadvantages, however, probably make the assay of tritium in the form of gaseous hydrogen or hydrocarbons more satisfactory. These disadvantages are the inconveniently large size of counter, the relatively small volume of water vapor it will hold, and the tedious decontamination procedure. Furthermore it seems likely to the present author that the recommended method of decontamination would be insufficient to restore the background to normal after the measurement of higher counting rates.

### Hydrogen Gas as a Medium for Tritium Assay

The preparation of tritium-hydrogen can be accomplished, as already noted, by the same methods as have been devised for the preparation of deuterium-hydrogen, although in some cases the



scale might have to be increased since up to 10 mM of gas are required in some instruments. In the following account of some of the methods of tritium assay described in the literature, not only the procedure for measuring tritium activity in hydrogen is given but also, when available, that recommended by the authors for preparing the hydrogen from water.

### IONIZATION CHAMBER METHODS

Several applications of ionization chamber techniques to the assay of tritium-hydrogen have been described. In many of the papers full details are not given because the techniques used are merely adaptations of existing ones devised for the gas phase assay of  $C^{14}$ . Thus Berstein, Bennett, and Fields (10) measure tritium by means of the ionization chamber-Lindemann electrometer method previously described by Janney and Moyer (11) for the routine assay of  $C^{14}$  in carbon dioxide. Biggs, Kritchevsky, and Kirk (12) use an ionization chamber with vibrating-reed electrometer which is very similar to that described by Brownell and Lockhart (page 53) for the gas phase assay of  $C^{14}$ . Tolbert (13) also uses ionization chambers with vibrating-reed electrometer for the routine measurement of both tritium and low-level  $C^{14}$  activities.

The application of ionization chamber techniques to the determination of tritium therefore very often involves only such modifications of the methods devised for  $C^{14}$  assay as are necessary for the generation of hydrogen and the filling of the chamber with an incondensable gas. The techniques described by Henriques and Margnetti and by Biggs, Kritchevsky, and Kirk are examples of how this may be done (6, 12).

#### *Method of Henriques and Margnetti*

*Reduction of water to hydrogen.* These workers (6) reduce their water samples with magnesium amalgam which has the advantage of effecting quantitative reduction at a much lower temperature ( $400^\circ$ ) than does magnesium alone ( $620^\circ$ ). The organic sample is first combusted and the water trapped in a U-trap ( $L$  in Fig. 57). The combustion technique used is not

described by Henriques and Margnetti, but any of those described in Chapter V which yield sufficient water could be used. If necessary the water obtained is diluted to give  $220 \pm 20$  mg.

The apparatus used for the reduction is shown in Fig. 57. About 2 gm. magnesium turnings and 3 gm. mercury are shaken

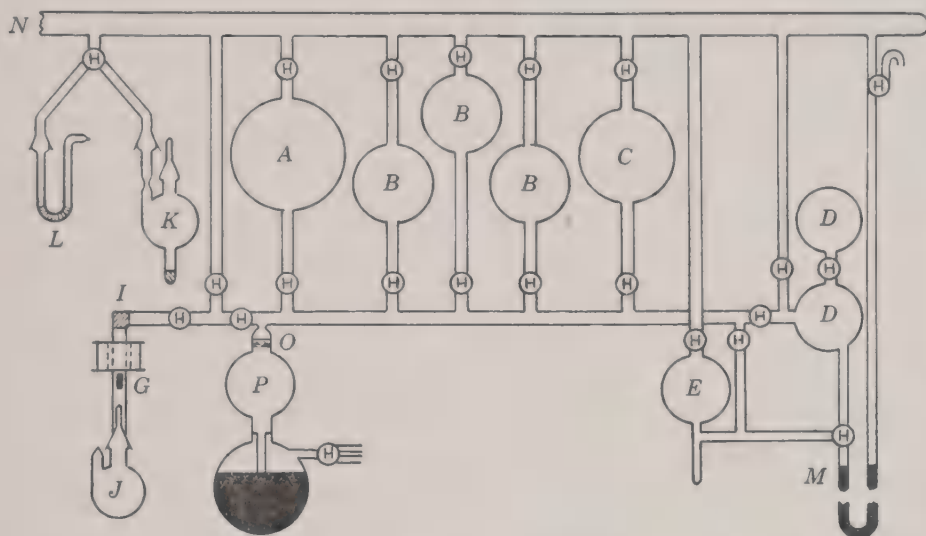


FIG. 57. Vacuum apparatus for tritium assay (Henriques and Margnetti).

A:  $H_2$  storage bulb (5 liters).

B: Storage bulbs (500 ml.)

C: Standard-sample storage bulb (1 liter).

D: Mixing chambers.

E: Ionization chamber (attached to electroscope; see Fig. 18).

G: Induction coil and hammer.

H: Vacuum stopcocks.

I: Glass wool plug.

J: Reaction flask in position to break seal.

K: Reaction flask with Mg/Hg amalgam.

L: U-tube with radioactive-water sample.

M: Manometer.

N: To mercury vapor pump.

O: Fritted disk of medium porosity.

P: Toepler pump.

together in a flask (K) which has a capacity of about 250 ml. The flask and U-tube L, which contains the water, are then attached by means of standard taper joints to the 3-way stopcock as shown in the figure. The water is frozen with a bath of liquid nitrogen, the apparatus evacuated, and the water sublimed into the reaction flask K, which is sealed off at the constriction. The contents

are well shaken to distribute the magnesium amalgam about the inside surface and the flask kept in the electric furnace at  $400^{\circ}$  for 1 hour. At the end of that time it is attached to the apparatus again, in the position indicated by the flask *J*, and opened by breaking the seal with the induction coil and hammer *G*. This releases the hydrogen into the manifold from which it is transferred to the ionization chamber by means of the Toepler pump *P*.

*Ionization chamber.* The ionization chamber and electroscope are similar to those already described on page 56 for the measurement of  $C^{14}$  activity in carbon dioxide. The apparatus depends for the measurement of radioactivity on the rate of discharge of a Lauritsen electroscope. When tritium is to be measured, certain minor modifications are made to the  $C^{14}$  apparatus. The silvered surface of the quartz flask is maintained at +125 volts instead of +240 volts, and the collecting wire, repelling post, and fiber are charged to -125 volts instead of -140 volts. These potentials are sufficient for the collection of all ions. Hydrogen gas has a very low ionization efficiency and samples of low activity therefore give very low discharge rates of the electroscope fiber. A more powerful eyepiece ( $20\times$  instead of  $6\times$ ) with closer graduations is therefore used.

*Filling the chamber.* Since hydrogen cannot be condensed into the chamber it would be difficult to introduce sufficient gas to exert a pressure greater than 1 atmosphere. High efficiencies are possible at 1 atmosphere, however, and pressures not much short of this can be obtained in the chamber by means of the Toepler pump. Thus after opening the reaction flask *J* (Fig. 57) the gas is transferred to the chamber *E* and the pressure adjusted to a standard value (70 cm.) as indicated on the manometer *M*. This ensures that the same amount of hydrogen is introduced into the ionization chamber each time. For this to be possible, however, at least 200 mg. water must be reduced. The vessel *A* contains inactive hydrogen for the measurement of background discharge rates. This inactive hydrogen could be used for adjusting the pressure in the chamber to the standard value if insufficient radioactive sample were available. It is more convenient, however, to dilute at the water stage. The vessel *C* contains a refer-

ence sample of radioactive hydrogen of known activity which is measured daily as a check on the apparatus. The vessels *D* are for mixing if for some reason dilution of gas samples becomes necessary.

Henriques and Margnetti tested this method by assaying the combustion water obtained from a sample of tritium-labeled benzene diluted with inactive benzene to a series of sub-dilutions. The average deviations of the observed activities from the calculated were in the range 0.7 to 1.4%. The method was found to be suitable for the measurement of about  $10^{-4}$   $\mu$ c. (200 disintegrations/min.) in 10 mM hydrogen. It is to be noted, however, that the method is somewhat slow, up to 1 hour being required for each determination.

*Method of Biggs, Kritchevsky, and Kirk*

These workers (12) describe a simple technique for the combustion of compounds doubly labeled with  $C^{14}$  and tritium and an apparatus in which the conversion of water to hydrogen and the filling of an ionization chamber is carried out. It is illustrated in Fig. 58. Trap A, formerly part of the combustion train, contains the water to be analyzed. It should amount to not less than 150 mg. The vessel B contains 3 ml. of a saturated solution of lithium aluminum hydride in anhydrous Carbitol. The apparatus is assembled as shown, the water frozen in liquid nitrogen, and the whole system evacuated through stopcock 2. When the lithium aluminum hydride solution is fully outgassed, stopcock 1 is closed, the water melted, and the vessel B rotated about the standard taper joint so that the contents are tipped into A. The hydrogen produced by the reaction is purified by passage through the liquid nitrogen chilled spiral trap. By means of the Toepler pump the hydrogen is then introduced into the ionization chamber which is attached to the apparatus in the position indicated in the diagram. The Toepler pump is operated by means of a leveling bulb and permits the pressure in the chamber to be adjusted to nearly 1 atmosphere. The activity is measured with a vibrating-reed electrometer and the rate of drift (see page 56) corrected to standard temperature and pressure.



Biggs and co-workers found no troublesome memory effects either in the glass apparatus or in the brass ionization chamber, provided all the apparatus was pumped out for 30 min. between each analysis.

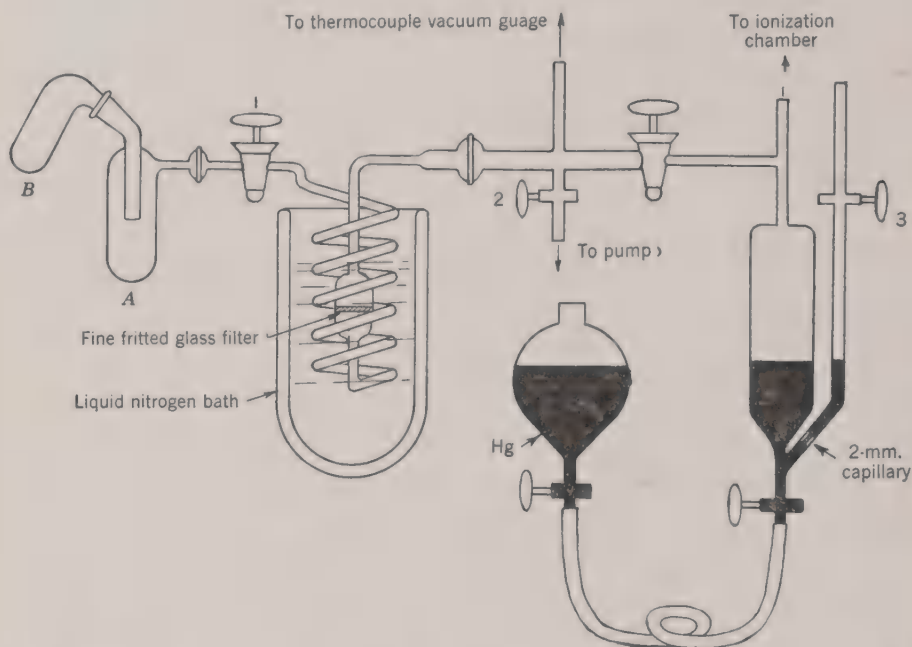


FIG. 58. Apparatus for generating tritium-hydrogen samples (Biggs, Kritchevsky, and Kirk).

### COUNTING OF HYDROGEN

The use in Geiger and proportional counters of hydrogen mixed with other gases and vapors is described by various authors, but the multiplicity of recipes for filling mixtures suggests that none of them is very satisfactory. Thus Kamen (14) recommends a mixture consisting of hydrogen at 25 cm. and ethanol vapor at 1.5 cm. mercury pressure, whereas Reid (15) uses a mixture of hydrogen at 9 cm., helium at 5 cm., and ethanol vapor at 1 cm. pressure. These mixtures are counted in the Geiger region. Other workers, notably Bernstein and Ballentine (4), use proportional counting and recommend hydrogen mixed with about 20 times its own volume of methane. Few of these workers, however, give precise details of counter characteristics and it is therefore diffi-

cult to judge their relative merits from the published accounts. The application of many of these methods might therefore be difficult, and readers who have particular reasons for wanting to count tritium in the form of hydrogen would be well advised to use the proportional counting method of Bernstein and Ballentine. They have given ample details in their published work and their method has been successfully used by other workers, notably Verly and co-workers (5).

#### *Proportional Counting Method of Bernstein and Ballentine*

These workers (4), whose method for the proportional counting of a carbon dioxide-methane mixture has already been described (page 66), have also counted radioactive hydrogen in the proportional region, again using methane as a quenching agent. Their filling apparatus is shown in Fig. 59 and is self-explanatory. The sample of tritium-hydrogen is contained in a 10-ml. vessel *A* provided with a stopcock and standard taper joint. This is attached to the apparatus and the system evacuated through the two-way stopcock *S*<sub>1</sub>. A portion of the hydrogen, sufficient to exert a pressure of about 4 cm. when in the counter, is then taken into the calibrated bulb *B* and transferred with the Toepler pump *D* to the counter *C*. The pressure in the counter is adjusted to 1 atmosphere with tank methane which is admitted through the two-way stopcock *S*<sub>1</sub>. Although the authors themselves do not specifically point out the fact, it is to be noted that the methane will sweep into the counter the tritium remaining in the dead space between stopcocks *S*<sub>3</sub> and *S*<sub>4</sub>. Thus all the sample measured out is transferred to the counter which is operated in the proportional region, as described for  $C^{14}O_2$  on page 66, and is found to give a satisfactory plateau.

The value of this method, unfortunately, is limited by counter contamination and the difficulties of getting rid of it. This was of the order of 12% after 1 hour's pumping, falling, however, to only 0.06% after prolonged pumping and flaming with the hand torch. The authors recognize that this is a serious obstacle to the use of their method and recommend the use of a hydrocarbon as a means of avoiding contamination.

Verly and co-workers (5) determine tritium by combustion, on an unspecified scale, and conversion of the water to hydrogen over hot zinc at  $400^{\circ}$ . The reduction is carried out in triplicate, the product of the first run being discarded to eliminate memory

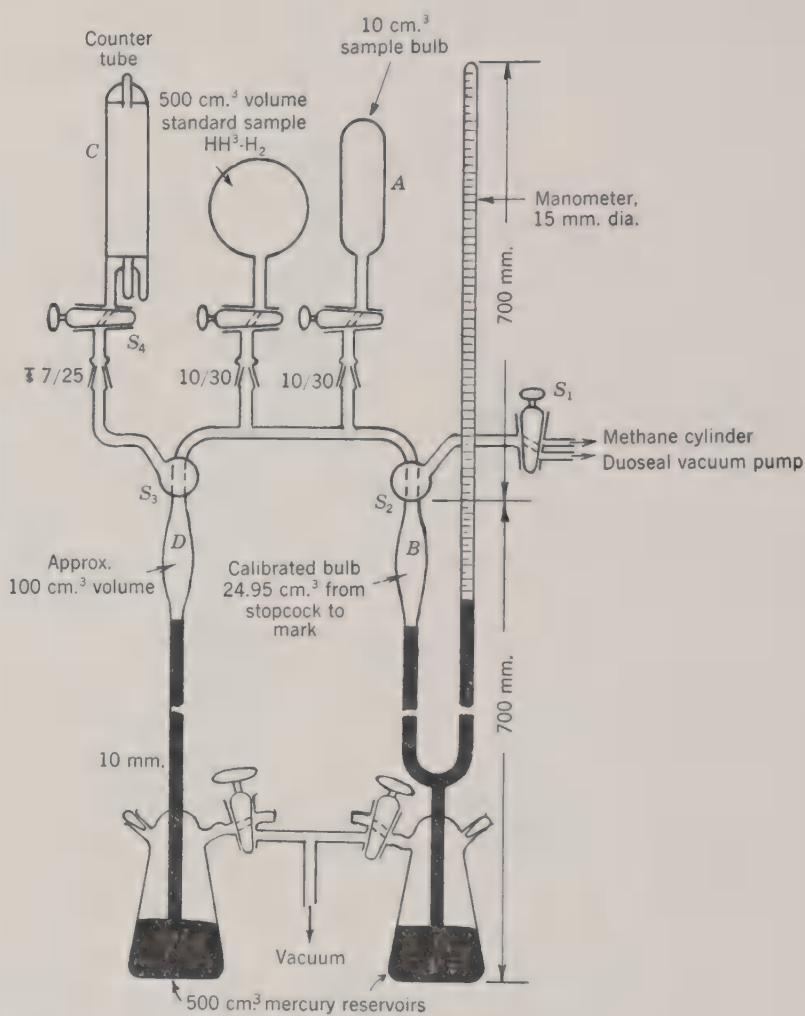


FIG. 59. Filling line for tritium samples (Bernstein and Ballentine).

effects in the reducer. The counter described is 18 mm. in diameter with a silvered cathode and an 0.002-in. tungsten anode wire. It is filled to 5 cm. pressure with the hydrogen sample to be assayed and the pressure adjusted to 70 cm. with methane. The counter is operated at 3400 volts, which corresponds with the

upper end of the proportional region. The electronic equipment includes a pulse amplifier and discriminator set to accept all pulses greater than 1 mv. The plateau of such a counter operated in this way was found to be about 800 volts with a slope of only 0.3% per 100 volts. Reproducibility was found from duplicate assays to be better than 1% using counting rates between  $10^4$  and  $5 \times 10^4$  counts/min. Unlike Bernstein and Ballentine, these workers mention no contamination of the counter, although, since both methods make use of very similar fillings, one might expect it to occur.

### Counting of Tritio-hydrocarbons

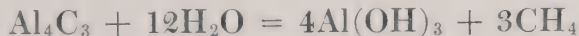
#### METHANE

Methane is known as a good counting gas and has been used, as already described, to confer stability on proportional counters containing carbon dioxide or hydrogen. Because it is so easily prepared from water it is an obvious choice as a medium for the assay of tritium in gas counters.

There are several ways of converting water to methane, and the methods used for the purpose of tritium determination make use of the reactions of water with aluminum carbide or with the Grignard reagent. In both methods the resultant methane is counted in a proportional counter.

#### *Method of White, Campbell, and Payne*

These workers (7) make use of the unique reaction between water and aluminum carbide which gives rise to methane



This equation indicates that half of the hydrogen atoms of water are transferred to methane, thus giving it half the activity of the water it is derived from.

It was found that, when water was reacted with aluminum carbide at room temperature, the resulting methane was contaminated with several impurities believed to consist chiefly of hydrogen, oxygen, ammonia, hydrogen sulphide, and unsaturated hydrocarbons. For this reason, probably, counters filled with the



gas prepared in this way were found to have no usable Geiger region.

*Generation of tritio-methane.* The reaction for the preparation of methane from water is carried out on the 200–300-mg. scale. It seems doubtful, in view of the method employed, whether it could be scaled down very much. The apparatus is shown in Fig. 60. The reaction vessel consists of the tube A which bears a stop-cock and standard taper joint. About 200–300 mg. of the water

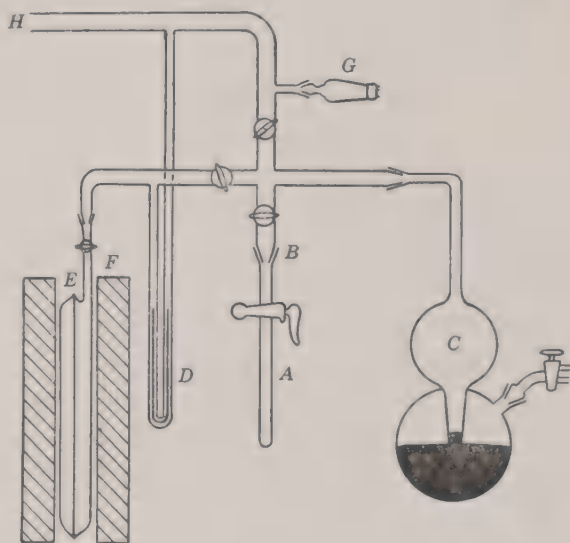


FIG. 60. Tritio-methane apparatus (White, Campbell, and Payne). A: reaction vessel; B: cone joint; C: Toepler pump; D: manometer; E: gas counter; F: 2-in. lead screen; G: Pirani gauge; H: pump line.

sample to be assayed is placed in it and frozen by immersing the lower end in a solid carbon dioxide bath. About 2 gm. finely powdered aluminium carbide is added and the tube, with the water still frozen, is attached to the vacuum line and evacuated to a pressure of  $10^{-2}$  mm. or less. The tube is then removed from the apparatus and heated at  $100^{\circ}$  for 1 hour, and the methane produced is partly purified by cooling the reaction vessel in a solid carbon dioxide bath for a further hour.

If the combustion technique devised by these workers has been used (see Chapter V) the water will be contained in tube

*M* of Fig. 39. The reaction vessel *A* of Fig. 60 is not then used; instead, the contents of tube *M* are frozen, aluminum carbide added, and the tube attached to the apparatus at *B* by means of the adaptor shown in Fig. 40.

*Counter.* The counter *E* used by these workers was a type GA 26 (20th Century Electronics) which consists of a 200-ml. tube with a graphite-on-glass cathode and tungsten-wire anode. It is fitted with a stopcock and standard taper joint. Any gas counter of similar construction would obviously do, but the large size is recommended for the purpose of counting large volumes and hence of measuring low specific activities.

The counter is attached to the apparatus, evacuated, and then, by means of the Toepler pump, filled to the required pressure (25 cm.) with the impure methane from the reaction vessel.

*Electronic equipment.* In the original work, the pulses from the counter, which was operated in the proportional region, were passed via a 0.001- $\mu$ f. capacitor to an amplifier with the gain set at 4000. The capacitor was a nonionizing type, other types having been found to give spurious pulses. The pulses were then passed to the scaler through a discriminator provided for the rejection of noise and pickup. The voltage applied to the anode of the counter was supplied from a high-voltage unit via a ripple filter and a 5-megohm anode load. At 25 cm. pressure of methane in the counter, however, the plateau was 200 volts long with a slope of 3% per 100 volts, and hence it was not necessary to stabilize the high voltage. This pressure, therefore, is the one recommended. It was found that tritium activities could be determined with a reproducibility of 2–3%. No contamination of the counter was observed although, if the gas contains hydrogen as an impurity, as suspected by Orchin, Wender, and Friedel (16), some contamination might have been feared.

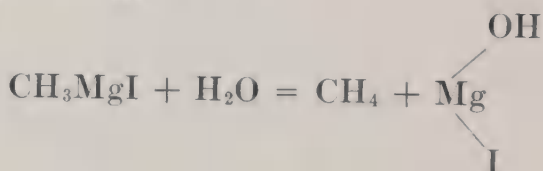
*Testing of method for isotopic fractionation.* The effect of reaction time upon the specific activity of the methane obtained from a standard radioactive water sample was investigated for times of between 10 min. and 16 hours. After heating the reaction mixture for 16 hours the specific activity of the gas was nearly 10% higher than that obtained after heating for only 10 min., sug-

gesting that tritium-containing water molecules react somewhat slower than those containing only hydrogen. The specific activity of the methane obtained after a reaction time of 1 hour, however, was not found to differ significantly from that obtained after a reaction time of 10 min.

A further investigation into possible fractionation was carried out by assaying a radioactive water sample first as hydrogen and then as methane. The hydrogen was produced by the reduction of water with magnesium amalgam according to the method of Henriques and Margnetti (see page 164) and then counted in admixture with methane as described by Bernstein and Ballentine (page 169). It was found that approximately 50% of the hydrogen in radioactive water passes into methane when allowed to react with aluminium carbide. Thus, although some slight fractionation can no doubt occur, it does not seem likely to introduce an appreciable error if the reaction time is kept always the same. A 1-hour reaction time is recommended because unreacted water is visible in the tube if it is heated for shorter periods.

#### *Method of Robinson*

Robinson (17) makes use of the well-known reaction between a Grignard reagent and water



which results in the transference of half of the water hydrogen atoms to methane. Providing no isotopic fractionation occurs, therefore, methane made in this way will have half the specific activity of the water it is derived from. In the published account of his method the author gives instructions for carrying out the reaction on the 250-mg. scale but does not state whether it would be possible to scale it down in the event of much less water being available. The present author has found, however, that it is difficult to promote the reaction between as little as 10 mg. water and a Grignard reagent dissolved in an immiscible solvent such

as butyl ether with anything like a satisfactory yield. It is probable therefore that Robinson's method, like that of White, Campbell, and Payne, is suitable only when there is a relatively large amount of water available.

*Generation of tritio-methane.* The apparatus is shown in Fig. 61. The reaction vessel 7, is a 100-ml. flask connected to a burette 6, in which the methyl magnesium iodide solution is kept. The side arm of the flask is covered with a vaccine port, 8. The flask is connected to the vacuum main 13, to the traps 9 and 11, and counter 12. The burette itself is also connected to vacuum so that its contents will flow into the flask under gravity alone, and it is protected from atmospheric moisture. A cold trap, 16, is inserted between the manifold and the vacuum pumps because the solvent used in the reaction (dibutyl ether) is appreciably volatile at room temperature. The progress of the reaction can be followed by means of the dial pressure gauge 17. The reaction flask is provided with a magnetic stirrer and is surrounded by a water bath.

*Procedure.* The flask, traps, and counter are thoroughly evacuated for at least an hour and the traps then cooled in liquid nitrogen. The magnetic stirrer is started up and boiling water poured into the bath surrounding the reaction flask. 8 ml. of 0.9 *N* methyl magnesium iodide dissolved in dibutyl ether are run into the flask from the burette and briefly exposed to the pumps to get rid of any dissolved gases that may have been released. 250 mg. water is then injected drop by drop into the flask through the vaccine port from a 1-ml. syringe. After 3 to 4 min. the pressure indicated on the gauge becomes steady, indicating that the reaction will go no further. The hot water around the flask is then replaced by a solid carbon dioxide bath and left for 5 min. This is to condense down the solvent, which has a vapor pressure of 4.8 mm. at 20° and would therefore distill appreciably at laboratory temperature. Stopcock 2 is adjusted to connect the flask with the spiral trap and, since methane has a vapor pressure of only 12 mm. at the temperature of liquid nitrogen, most of it condenses. It is next transferred to the U-trap 11 by opening stopcock 3 and removing the liquid nitrogen from around the spiral trap. Stopcock 3 is closed and the liquid nitrogen around the U-trap re-



placed by a solid carbon dioxide bath which permits the methane to vaporize into the counter but keeps any contaminating vapors frozen down. The gas in the counter is adjusted to a standard pressure of 389 mm. at 25° by pumping excess through stopcock

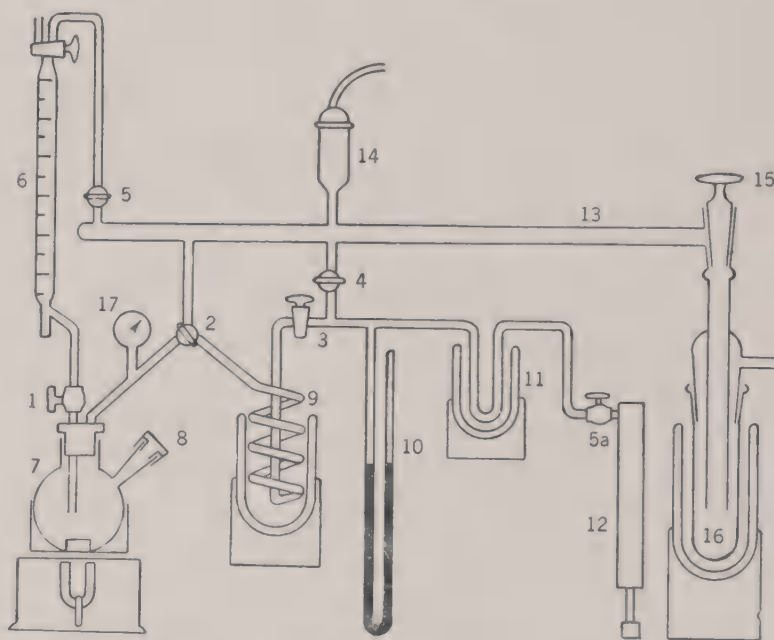


FIG. 61. Tritio-methane apparatus (Robinson).

- |  |                          |
|--|--------------------------|
| 1 and 5a: Hoke packless valves.          | 10: Manometer            |
| 2: 3-way 120° stopcock.                  | 11: U-trap.              |
| 3, 4 and 5: 2-way stopcocks.             | 12: Counter.             |
| 6: 100 ml. burette for Grignard reagent. | 13: Manifold.            |
| 7: 100 ml. reaction flask.               | 14: Thermocouple gauge.  |
| 8: Vaccine port.                         | 15: Main stopcock.       |
| 9: Drying coil.                          | 16: Vapour trap.         |
|  | 17: Dial pressure gauge. |

4, and the counter is attached to the electronic apparatus for counting.

Robinson found that, under the conditions described, the reaction went to 85% completion, calculated on the methyl magnesium iodide. Thus 14 millimoles of water reacted with 7.2 millimoles of methyl magnesium iodide to give 6.1 millimoles of methane. This, however, is only 43% calculated on the water. It

was found that a smaller excess of water could not be used without reducing the yield of methane, and this presumably represents the best yield obtainable. When as much as 250 mg. water is available, however, a low yield does not matter, provided it is not accompanied by isotopic fractionation. Robinson found that if the reaction was carried out at  $0^{\circ}$ , instead of at nearly  $100^{\circ}$ , the specific activity of the methane obtained from a standard water sample was lowered by about 20%. When the reaction is carried out as recommended, however, he claims that reproducible results are obtained and that the specific activity of the methane is always proportional to that of the water.

It is to be noticed that the sample is taken for counting by filling the counter to a standard pressure and discarding the excess gas. Although methane is partly condensed at the temperature of liquid nitrogen, it is still not possible to measure known volumes of gas accurately in a calibrated manometric chamber and to transfer them quantitatively to the counter. Hence only one sample from each methane preparation can be counted. White, Campbell, and Payne also completely fill their counters with the sample to be assayed. In the present author's opinion, a material increase in the flexibility and accuracy of both methods would be achieved by the use of some measuring device such as that described by Bernstein and Ballentine for the measurement of hydrogen samples (see Fig. 59). In the apparatus recommended by White, Campbell, and Payne, the Toepler pump could itself be calibrated like a McLeod gauge for the measurement of very small volumes. A reservoir of inactive methane would have to be incorporated into the apparatus for the adjustment of pressures in the counter. Such modifications of both sets of apparatus would make possible the sub-division of the tritio-methane into several portions. This arrangement would not only be of advantage when the gas has an inconveniently high specific activity, but it also makes possible the assay of replicate samples with a corresponding gain in accuracy.

A further interesting feature of Robinson's technique lies in his preference for metal apparatus. His counter is constructed entirely of metal with a packless valve replacing the usual glass

stopcock. In a footnote to his paper he records that a further development is a vacuum apparatus made mostly of metal and using oil instead of mercury (mercury vapor being thought to interfere with the operation of his counters). It is, of course, a matter of opinion whether glass or metal apparatus is preferable for vacuum work, but certainly not everyone would agree that a change from glass to metal apparatus was a change for the better.

#### ASSAY AS HYDROGEN-METHANE MIXTURES

##### *Method of Wilzbach, Kaplan, and Brown*

A very useful method for the quantitative conversion of tritium in organic components to a gas mixture in a single operation has been described by these workers (18). The conversion is carried out in Pyrex reaction tubes approximately 17 cm. long, 1 cm. in diameter, and provided with breakseals. They must be made of special heat-resistant glass (Pyrex 1720, Corning Glass Works) as they have to withstand a temperature of over 600°. Each tube is charged with 1 gm. 30-mesh zinc powder, 100 mg. nickelic oxide ( $\text{Ni}_2\text{O}_3$ ), and 5–6 mg. water. A 5–10-mg. sample of the material to be assayed, contained in a porcelain boat if solid or in a fragile glass ampule if liquid, is then added and the tube evacuated and sealed. It is agitated to break the ampule and mix the contents and heated at  $640^\circ \pm 10^\circ$  for three hours. By this treatment, the tritium in the compound is converted to a mixture of hydrogen and methane. The tube is then attached to a suitable vacuum manifold, opened, and the gaseous contents withdrawn and introduced into the instrument in which its radioactivity is to be measured. Wilzbach, Kaplan, and Brown use an ionization chamber with vibrating-reed electrometer but consider that the gas mixture should be equally suitable for use in a gas counter. As a test of the method they applied it to the assay of a considerable range of labeled compounds, including radioactive water itself, and found that their results agreed closely with those obtained when the compound was assayed by combustion and conversion of the water to hydrogen.

The most important advantage of this method is that a gas suitable for assay is obtained in a single operation whereas all other published methods involve combustion and subsequent conversion of the combustion water to some other compound. Another advantage is that the reaction is carried out in expendable apparatus, thus eliminating the danger of memory effects. Its only obvious disadvantage is the failure to separate carbon and hydrogen, and hence samples containing both  $C^{14}$  and tritium cannot be assayed.

## REFERENCES

1. M. L. EIDINOFF and J. E. KNOLL, *Science* **112**, 250 (1950).
2. F. N. HAYES and R. G. GOULD, *Science* **117**, 480 (1953).
3. W. J. ARROL and E. J. WILSON, private communication.
4. W. BERNSTEIN and R. BALLENTINE, *Rev. Sci. Instr.* **21**, 158 (1950).
5. W. G. VERLY, J. R. RACHELE, V. DU VIGNEAUD, M. L. EIDINOFF, and J. E. KNOLL, *J. Am. Chem. Soc.* **74**, 5941 (1952).
6. F. C. HENRIQUES, JR. and C. MARGNETTI, *Ind. Eng. Chem. Anal. Ed.* **18**, 420 (1946).
7. D. F. WHITE, I. G. CAMPBELL, and P. R. PAYNE, *Nature* **166**, 628 (1950).
8. R. F. GLASCOCK, *Nucleonics* **9**, No. 5, 28 (1951).
9. N. PACE, L. KLINE, H. K. SCHACHMAN, and M. HARFENIST, *J. Biol. Chem.* **168**, 459 (1947).
10. I. A. BERSTEIN, W. BENNETT, and M. FIELDS, *J. Am. Chem. Soc.* **74**, 5763 (1952).
11. C. D. JANNEY and B. J. MOYER, *Rev. Sci. Instr.* **19**, 667 (1948).
12. M. W. BIGGS, D. KRITCHEVSKY, and M. R. KIRK, *Anal. Chem.* **24**, 223 (1952).
13. B. M. TOLBERT, private communication.
14. M. D. KAMEN, "Radioactive Tracers in Biology," Academic Press Inc., New York, 1947.
15. A. F. REID, "Preparation and Measurement of Isotopic Tracers" (D. W. Wilson, A. O. C. Nier, and S. P. Reimann, eds.) p. 104, J. W. Edwards, Ann Arbor, Michigan, 1946.
16. M. ORCHIN, I. WENDER, and R. A. FRIEDEL, *Anal. Chem.* **21**, 1072 (1949).
17. C. V. ROBINSON, *Rev. Sci. Instr.* **22**, 353 (1951).
18. K. E. WILZBACH, L. KAPLAN, and W. G. BROWN, *Science* **118**, 522 (1953).



## CHAPTER IX

### THE DETERMINATION OF TRITIUM (CONTINUED)

#### Assay as Tritio-butane Using Permanent Vacuum-line Technique

In the last chapter several methods for the gas phase assay of tritium were described. Although one method for the counting of water vapor itself is available, most workers in this field have preferred to use either elemental hydrogen or methane. Neither of these gases is entirely satisfactory. Hydrogen has poor counting properties in both the Geiger and proportional regions, unless mixed with more than ten times its own volume of methane to increase counter stability; it is not condensed at the temperature of liquid nitrogen and consequently the measurement and quantitative transfer of small samples can be accomplished only by means of a Toepler pump, which is tedious and time consuming; hydrogen contaminates counters under the influence of the discharge, thus causing memory effects which are difficult to eliminate.

Methane has much better counting properties than hydrogen, especially in the proportional region. Good plateaus are obtained from counters filled with methane at pressures up to 40 cm. and it does not contaminate counters by exchange. Like hydrogen, however, it suffers from the disadvantage of not being fully condensed at the temperature of liquid nitrogen.

Methods are available for the generation of hydrogen from water on a scale of 5–10 mg., which is convenient for most biological purposes. (See methods of Graff and Rittenberg, page 147, and of Dubbs, page 149, for the preparation of deuterium-hydrogen.) Attention must be paid, however, to the dangers of contaminating the reduction apparatus. As far as is known to the

present author, the only methods available for the generation of methane from water are the two described in the last chapter, and both of them are designed for the 250–300-mg. scale. From a consideration of the techniques used it seems doubtful if they could be scaled down very easily. They may not, therefore, always be applicable to the determination of tritium in the small samples of material that it is usually possible to isolate in biochemical experiments.

The assay of tritium as tritio-butane was devised by the present author to meet certain requirements which are met by none of these other methods. These were for a gas which could both be readily prepared in good yield and without isotopic fractionation from water and be fully condensed at the temperature of liquid air. Thus vacuum techniques, and indeed vacuum apparatus, designed for  $C^{14}$ -gas counting could be used also for tritium, with a correspondingly smaller demand than might otherwise have been made on the capabilities of assistant staff. Further, a gas which could be counted in the Geiger region was preferred not only because Geiger counting necessitates less electronic equipment than does proportional counting, and this equipment is expensive, but also because such equipment must be maintained, and the less maintenance required the better. The final requirement was for a gas which could be prepared on a scale of 5–10 mg. because, in the work envisaged, not much more water than this was likely to be obtainable. This point is particularly important; if necessary the manipulation of incondensable gases can be undertaken, even if inconvenient, and a large institution can afford the purchase and maintenance of as much electronic equipment as is needed. But the yield of metabolites from many biochemical experiments often cannot be much increased without very great difficulty.

In choosing a gas into which water can be converted, it was decided to use one of the hydrocarbons for reasons discussed in the last chapter. *Normal* butane was found to have excellent physical properties for the purpose required; it is fully condensed at liquid air temperature and has a vapor pressure of about 13 mm. at  $-78^{\circ}$ . This means that it can be purified from less volatile

substances such as water by simply distilling through a U-trap cooled in a solid carbon dioxide bath. There was also reason to hope that it would be a useful counting gas in the Geiger region because Powell and Reid (1) had actually counted it in the course of an investigation into the isomerisation of butanes but had not given details of plateau length and slope.

#### PREPARATION OF TRITIO-BUTANE FROM WATER

In this technique (2) butane is produced from the reaction between water and butyl magnesium bromide. Although the same in principle the method differs in several important details from that of Robinson who uses the reaction between water and a Grignard reagent to produce methane. His method of tritium determination, described in detail on page 174, is designed for the 250-mg. scale. Calculated on the Grignard reagent present, his yield of methane is 85% corresponding to 43% calculated on the water, since this is the reactant present in excess. The method of producing butane described here, however, is carried out on the 10-mg. scale, and the Grignard reagent, which is used dry and not in solution, is the reactant present in excess. Yields, calculated on the water, are then nearly quantitative when the Grignard reagent is freshly prepared. Although the yield diminishes as the reagent gets older, the specific activity of the butane obtained from a standard water sample remains the same, indicating that, as in Robinson's method, no isotopic fractionation occurs.

#### *2 N Butyl Magnesium Bromide*

Although most chemists feel confident of their ability to prepare a simple Grignard reagent, a short account is included here. This is chiefly because good yields of butane and low blanks depend on the purity of the reagent, and the following technique has been found satisfactory.

*Apparatus.* The apparatus, all of which is fitted with standard taper joints, consists of a 100-ml. flask fitted with a Claisen head bearing a reflux condenser and dropping funnel. When not in use it is kept in the oven at 100° and assembled just before use



with all openings closed with calcium chloride tubes. After use it is washed thoroughly and returned to the oven.

*Reagents.* Sodium-dried ether must be thoroughly dry. Technical ether is stored over sodium for about a week and then decanted on to fresh sodium and kept for at least another week before use. If the ether is not thoroughly dry, the butyl bromide does not react with an equivalent of magnesium and high blanks are obtained.

*The normal butyl bromide* is carefully redistilled and a middle cut at constant boiling point collected. The *magnesium turnings* should be Grignard reagent quality. Some samples of magnesium with this description have been found to yield Grignard reagents containing a considerable amount of black sludge. Such magnesium is unsuitable.

*Procedure.* Each tritium determination consumes about 1.5 ml. of 2 N reagent, and the reaction tubes are conveniently prepared in batches of six. Thus 30 ml. of reagent is enough for 18 tubes. The reagent deteriorates on keeping and should not be used when more than a week old. Although there is no reason why six determinations a day should not be carried out if required, 18 a week is a fair average and will keep one worker fairly well occupied. Batches of 30 ml. of reagent prepared once or twice weekly are therefore convenient.

First, 8.2 gm. redistilled butyl bromide are accurately weighed into the dropping funnel and 1.4 gm. pure magnesium turnings weighed into the flask. Then 30 ml. dry ether are measured into a dry stoppered measuring cylinder and a sufficient quantity poured into the flask to cover the magnesium. About 2 ml. butyl bromide is then run in. The flask is gently warmed to start the reaction and the remainder of the ether added to the butyl bromide in the dropping funnel. The solution is allowed to drop in at a speed sufficient to keep the reaction going briskly. When it has all been added, the solution is refluxed for a further hour on the steam bath. The product is slightly opalescent with little or no unreacted magnesium. The flask is disconnected, closed with an ungreased glass stopper, and stored in a desiccator containing phosphorus pentoxide.



*Preparation of Reaction Tubes*

The reaction tube consists of a 13-ml. tube with standard taper joint to which a small stopcock with a cone and socket can be attached. The total space beneath the stopcock is then about 18 ml. This is just about enough space to accommodate the butane theoretically obtainable from 10 mg. water without the production of an excessively high pressure at the temperature of the reaction. Three or four sets, each consisting of six units, are made, stopcocks and tubes being numbered so that the same pair is always used together. All tubes and stopcocks must be scrupulously clean and dry. Tubes that have been used and contain partly reacted Grignard reagent are washed with tap water, cleaned with chromic acid cleaning fluid, rinsed with distilled water, dried in the oven, and kept in a desiccator until required. Stopcocks, especially if they have been used with very active samples, are cleaned according to the procedure recommended by the manufacturers of silicone high-vacuum grease; viz., degrease with solvent, clean with dilute alkali, and boil with chromic acid. They are then rinsed, dried, and stored in the desiccator.

Each tube of reagent is prepared by the following procedure: The stopcocks are greased with silicone high-vacuum grease and a smear of the same grease is applied to the cone. A small wad of oven-baked glass tape ( $1 \times \frac{1}{2}$  in.) is rolled up and inserted into the tube beneath the stopcock to protect it from splashes when the ether is distilled off. This tape must have been previously heated to a temperature sufficient to destroy organic matter, or high blanks will result. 1.5 ml. of 2N butyl magnesium bromide solution is then rapidly measured into each reaction tube, an assistant attaching each one to its stopcock as soon as it is charged with reagent. This minimizes the exposure of the reagent to air.

The contents of each tube are then frozen by immersion in liquid air and the tube attached to the apparatus, as shown in Fig. 62, and evacuated. As a high vacuum is not essential for this part of the procedure, the joint *J* need not be greased. The ether is then distilled off with gentle warming and rotating of the tube to distribute the reagent as a thin layer. By having more attach-

ment points to the ether trap, more tubes can be treated at a time, and, with practice, six tubes can be treated in as many minutes. When the ether has been removed from all the tubes they are attached to the manifold shown in Fig. 63 and baked under open pumps for 2 hours at  $120^{\circ}$ . For this purpose a small asbestos or sheet-metal oven just large enough to accommodate all the reaction tubes up to their stopcocks is used. It is convenient if the oven is fitted with a boss-head by means of which

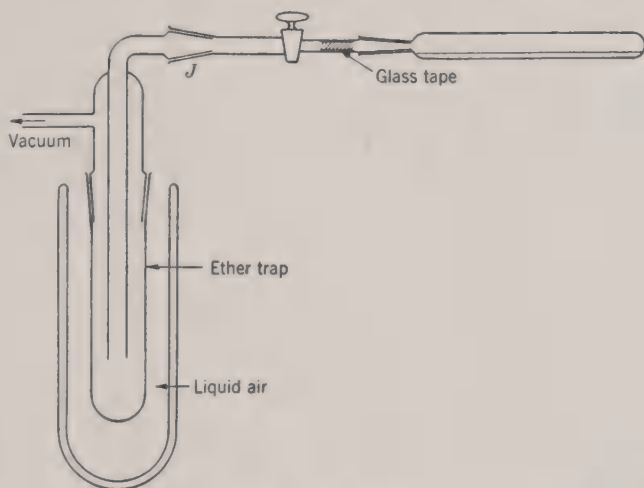


FIG. 62. Preparation of dry Grignard reagent, showing auxiliary apparatus attached to rough vacuum for removal of ether from solution of 2 *N* butyl magnesium bromide and deposition of dry reagent in reaction tube.

it is clamped to the main scaffolding. The input can be controlled with a constant-energy control (Simmerstat) or a variable transformer. Temperatures higher than  $120^{\circ}$  damage the reagent in some way and reduce the yield of butane obtained when treated with water; temperatures lower than  $110^{\circ}$  result in high blanks. After baking, the blank value may be measured by closing the stopcocks and baking for another hour at the same temperature. The gaseous contents of each tube are then transferred to the McLeod gauge  $G_1$  and measured. The gas obtained usually amounts to about  $100\ \mu\text{l}$ . and seldom to more than  $150\ \mu\text{l}$ . If this same volume of "blank" gas is present in the butane (theoret-

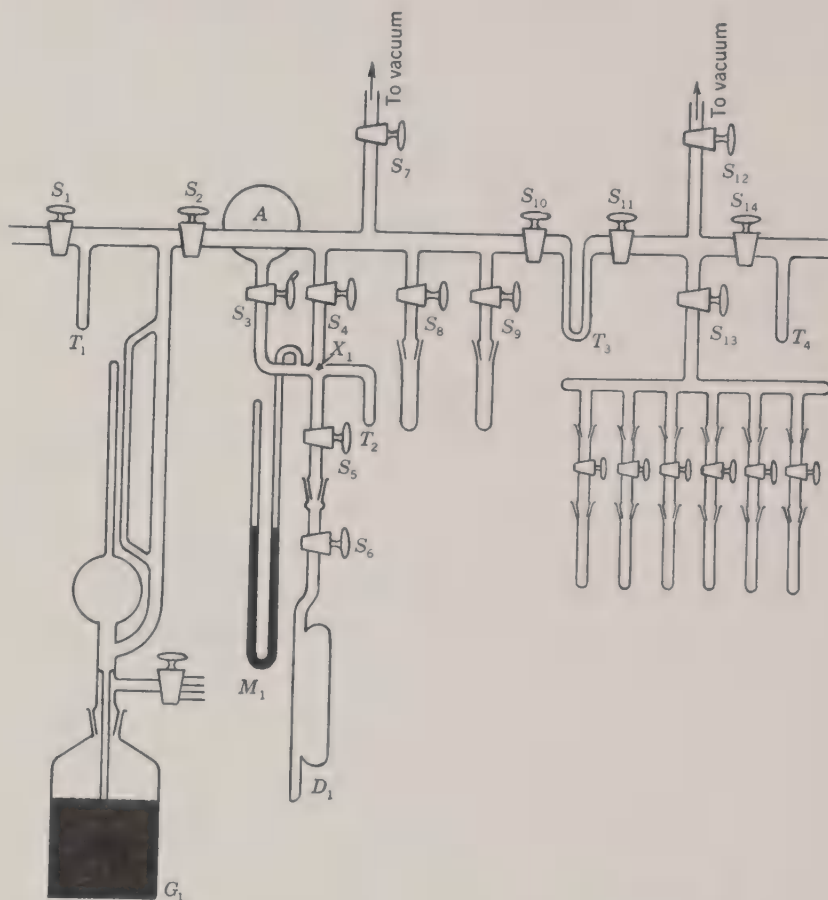


FIG. 63. Apparatus for the preparation and measurement of tritio-butane and its introduction into gas counters (part of permanent vacuum line shown in Fig. 1).

A: Butane reservoir.

D<sub>1</sub>: Stainless-steel-cathode gas counter.

G<sub>1</sub>: McLeod gauge.

M<sub>1</sub>: Manometer.

S<sub>1</sub>–S<sub>14</sub>: Stopcocks.

T<sub>1</sub>–T<sub>4</sub>: Traps and condensation tubes.

X<sub>1</sub>: Manometric space.

ically, 12.4 ml.) produced from 10-mg. samples of water, then the observed specific activity will be too low by about 1%. In the author's laboratory, the occasional tube that yields a blank of more than 150  $\mu$ l. is rejected. If, however, an error greater than about 1.5% could be tolerated, then such a tube could obviously be used.

### *Reaction with Water*

A weight of sample which will yield about 10 mg. water is combusted in the manner described in Chapter VI and the carbon dioxide separated by sublimation at  $-78^{\circ}$ . A reaction tube is then cooled in liquid air and the water condensed into it. The stopcock is closed and the tube heated for 1 hour at  $120^{\circ}$ , either with a small cylindrical oven of the type recommended for baking counters (page 81) or with the main oven, already mentioned, if several tubes must be baked at a time. At the end of that time the stopcock is opened and, with the heater still around the tube, the gaseous contents are extracted over a period of 5 min. by distilling through a U-trap ( $T_3$ ) cooled in a solid carbon dioxide bath. This holds back any water which may have escaped reaction. Although in the course of some 600 assays no unreacted water has ever been detected, this is thought to be a worth while precaution because radioactive water would not only have twice the specific activity of the butane and introduce an error in that way but it would also contaminate the counter.

If the sample to be assayed is not an organic compound but is already in the form of water, a 10-mg. sample is sublimed *in vacuo* into a reaction tube. The tube should not be opened for the purpose of adding the sample from a pipette as this procedure will expose the reagent to atmospheric moisture.

The butane is collected in the manometric chamber  $X_1$  and portions taken for counting exactly as described for carbon dioxide in Chapter VI, except that no carbon disulphide is needed. The McLeod gauge  $G_1$  is used for the measurement of small volumes when the butane sample has a high specific activity.

*Purity of the butane.* The mass spectrum of butane prepared by this reaction has been compared with that of pure normal butane and also with that of a 6% ether-butane mixture. It is calculated that the "synthetic" butane contains less than 0.04% ether. The spectrum of the gas produced in the "blank" indicates it to be chiefly *butene* and not ether or even butane as might be expected. The reactions responsible for the presence of this substance have not been investigated.



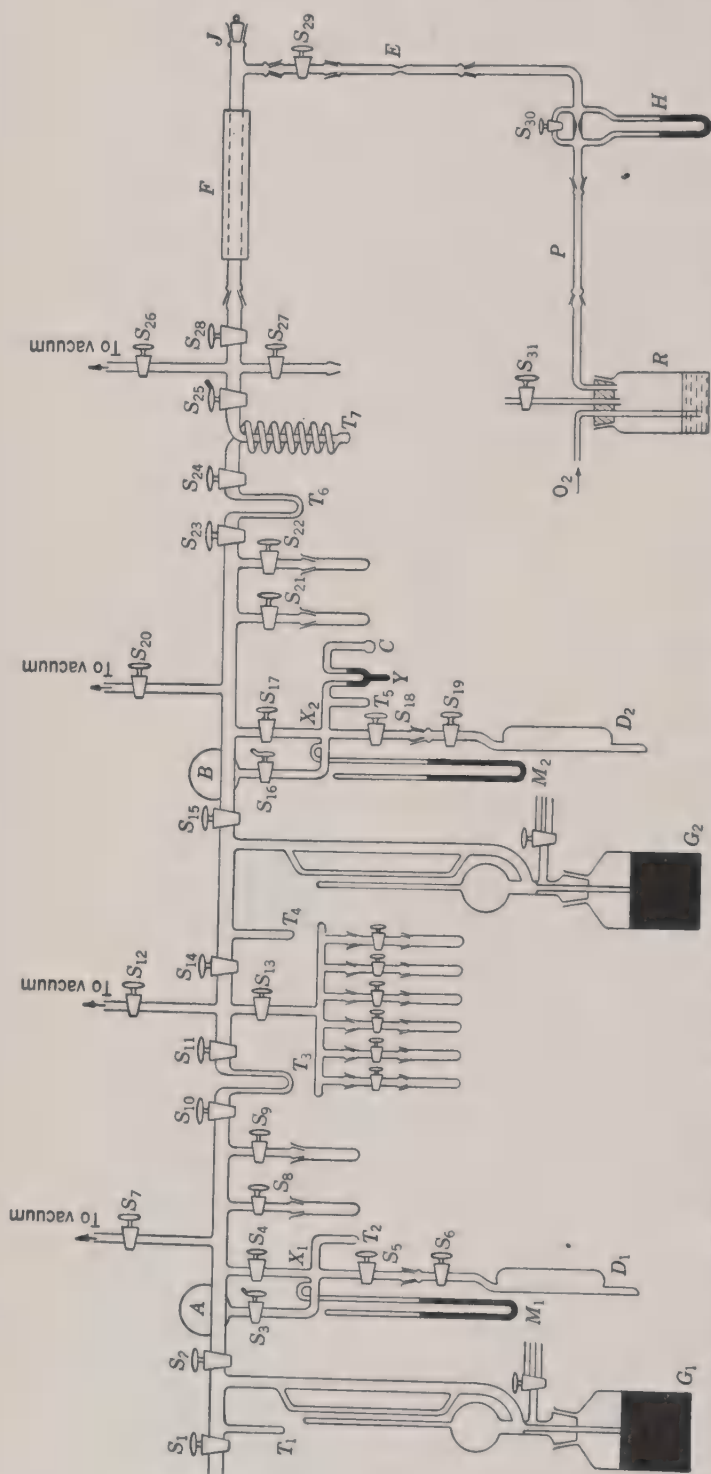


FIG. 1. Permanent high-vacuum line, showing lay-out of apparatus recommended by the author for the determination of tritium,  $C^{13}$ , and  $C^{14}$  in a single, 10-mg sample.

A: Butane reservoir

B:  $CO_2$  reservoir

C:  $CS_2$  reservoir

D<sub>1</sub>: Stainless-steel-cathode gas counter

D<sub>2</sub>: Graphite-cathode gas counter

E: Oxygen purification tube

F: Furnace and combustion tube

G<sub>1</sub> and G<sub>2</sub>: McLeod gauges

H: Flowmeter

J: Standard B14 taper joint and stopper

M<sub>1</sub> and M<sub>2</sub>: Manometers

P: Platinized asbestos tube with B10 joints

R: Bubbler and oxygen reservoir

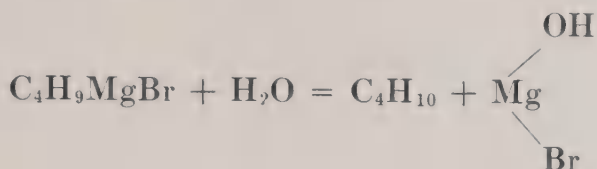
S<sub>1</sub>-S<sub>31</sub>: Stopcocks (S<sub>26</sub> is "control stopcock," see text and Fig. 6b)

T<sub>1</sub>-T<sub>7</sub>: Traps and condensation tubes

X<sub>1</sub> and X<sub>2</sub>: Manometric chambers

Y: Mercury cut-off (see Fig. 13).

*Factors affecting the yield.* If the ethereal solution of butyl magnesium bromide and the tube of dry reagent obtained from it are both freshly prepared, the yield of butane is 95–100% of that expected from the reaction



i.e., 12.4 standard ml. butane from 10 mg. water.

Aging either of the solution or of the dry reagent causes a reduction in yield, but unfortunately it has not been possible to investigate all the possible causes for this observation; it constitutes a substantial research problem in itself and one which has not been systematically investigated because the reaction, which is used primarily for analytical purposes, has been found to yield reliable results. The following facts have emerged, however, in the course of experience. When weighed samples of water are heated with the dry reagent about two weeks old, the butane obtained by extracting the hot tube in the usual way for the standard 5 min. may be as little as 60% of theoretical. If the tube is extracted by keeping the heater in position and removing gas as it is evolved for a further 15 min. about 20% more gas may be recovered. No unreacted water has ever been detected in the gas, and radioactive measurements have shown that the later fractions have the same specific activity as the first main fraction. It seems likely therefore that the extra butane obtained on further extraction is recovered from physical combination—possibly from a state of solid solution or adsorption. The fact that the last fraction has the same specific activity as the first main fraction suggests that the reaction has already gone to completion when the tube is first opened and that the extra gas is not the result of a further reaction. As the freshly deposited reagent does not retain butane in this way it seems likely that some change in the physical nature rather than in the chemical reactivity of the dry reagent occurs on standing.

The effect of repeatedly opening the flask of ethereal solution is to increase the size of the blanks. The dry reagent prepared from a solution two weeks old which has been frequently opened for the withdrawal of a few milliliters at a time may yield blanks as high as 200  $\mu$ l. each. This is presumably due to absorption of water vapor from the air although it is to be noted that the blank is obtained by heating for an hour after previously baking for two hours on open pumps when any butane contained in the reagent would be expected to disappear.

#### BUTANE-FILLED COUNTERS

As already noted, Powell and Reid in 1945 had reported butane to be a good counter gas but gave only scanty details. The first counter tried in the present author's laboratory for the purpose of counting butane was a graphite-cathode gas counter originally intended for  $C^{14}O_2$  assay. When filled with butane at 14 cm. pressure this counter was found to have an excellent characteristic in the Geiger region with a long flat plateau extending over about 800 volts with a slope of about 3% per 100 volts. Other graphite-cathode counters, all equally satisfactory for counting the standard carbon dioxide-carbon disulphide filling, were useless when filled with butane and showed no Geiger region at all. Other cathode materials were therefore tried, first passive iron as used in halogen-quenched counters and later "Staybrite" stainless steel. Some of the passive-iron-cathode counters—perhaps two or three in five—gave good characteristics when filled with butane, but the proportion of failures was still inconveniently high. The stainless-steel-cathode counters, however, have proved excellent, the number of failures being no greater than is found among the graphite-cathode counters used for  $C^{14}$  counting—i.e. 10–15%.

The life expectancy of this type of counter is not known. Theoretically it might be expected to be finite owing to the deposition of hydrocarbon polymers on the electrodes. It has been suggested that this is the cause of failure of ethylene-quenched counters (3). Some butane counters in the present author's laboratory, however, have been in continuous daily use for 12 months and are still satisfactory.

The recommended dimensions of the counters are those already described for  $C^{14}O_2$  counting; that is to say they should be made from glass tubing 16 cm. long and 27–30 mm. in diameter. The recommended working gas pressure is 14 cm., operating at a threshold voltage of about 3200 volts. The same precautions against electrical leakage therefore need to be taken for butane counters as for carbon dioxide counters; they should not be made too narrow (minimum outside diameter, 2.5 cm.) and the glass around the electrodes should be treated with silicone.

A new counter is put into commission by attaching a stopcock with standard taper joint, applying silicone solution, and baking out. Baking is less essential than for graphite-cathode counters and treatment for a few minutes with a hand torch is often sufficient, provided care is taken not to heat the tungsten-to-glass seals too suddenly.

*Counter filling.* The counter is attached to the high-vacuum apparatus at the joint below stopcock  $S_5$  (Fig. 63) and evacuated. For the measurement of background, pure *n*-butane at a pressure of 14 cm. is added from the reservoir A. If a radioactive sample is to be counted, stopcock  $S_5$  is closed after evacuating the counter, and the sample is condensed into the manometric chamber  $X_1$  and measured in the usual way. It is then allowed to expand into the counter and the pressure adjusted with inactive butane to the value previously found to be exerted when a counter-full of gas at 14 cm. is shared between counter and manometric chamber. The whole of the gas is then condensed into the counter, the stopcocks are closed, and the counter detached from the apparatus.

### *Electronic Equipment*

This is similar to that already described for  $C^{14}O_2$  counting and consists of a preamplifier, quenching unit, high-voltage unit, scaler, and oscilloscope. The pulses from the counter, as recorded on the oscilloscope screen, are not all of uniform size, some being about half and others about twice the average. The counter characteristic is usually satisfactory, however, with a plateau of 400 volts or more and a slope of less than 3% per 100 volts.



## REPRODUCIBILITY OF ASSAYS

The reliability of the method was first tested by assaying a standard solution of radioactive water and various sub-dilutions of it. The standard solution contained a nominal  $2 \mu\text{c./ml.}$  of tritium and yielded butane counting at 1530 counts/min./ml. gas. Since only half the tritium in the water is transferred to the butane,  $2 \mu\text{c./ml.}$  water corresponds with 1775 disintegrations/min./ml. in the butane. The observed activity of 1530 counts/min./ml. gas therefore implies a counter efficiency of 86.5% if the nominal activity of the radioactive water was its true activity. Sub-dilutions of this water were also assayed with the results shown in Table 6 where it will be seen that there is good agreement between the observed and calculated activities.

TABLE 6

Water sample (% stock)	Vol. butane assayed (ml.)	Activity (Counts/min.)	Specific activity (Counts/min./ml.)		Error (%)
			Obs.	Calc.	
10	8.85	1,353	153	153	0
20	3.70	1,152	312	306	+2.0
40	2.59	1,554	600	612	-2.0
60	{ 1.005 0.860	{ 908 802	915	918	-0.33
80	0.805	972	1,210	1,224	-1.7
100	0.645	987	1,530	—	—

Since that particular test was carried out, many samples have been assayed in replicate and it has been found that reproducibility is usually better than  $\pm 2\%$ . It is thus certainly as good as that observed for  $\text{C}^{14}\text{O}_2$  counting provided the recommended precautions against memory effects are taken.

Because pure *isobutane* is more easily obtained in England than is *n-butane*, an attempt has been made to use this method to prepare tritio-*isobutane*. Although *isobutane* is just as good a counting gas as the *n*-isomer, persistently high blanks ( $500 \mu\text{l.}$

and more) have been obtained from *isobutyl* magnesium bromide. The reason for this finding has not been discovered but at the time of writing the author is confident of satisfactory results only if *n*-butyl magnesium bromide is used.

*Memory effects.* No serious memory effects are observed in the apparatus, and those that do occur are chiefly because of the fact that butane, like other hydrocarbons, is slightly soluble in vacuum grease. Very high specific activities should therefore be avoided if possible and, where highly active butane has been used, the stopcocks and standard joints that it has been in contact with should be cleaned and re-greased immediately. This procedure should be followed by monitoring the whole of the section used for manipulating butane; a stainless-steel-cathode counter is filled with inactive butane to a pressure of 14 cm., its background counting rate is measured, and it is then re-attached to the apparatus. The inactive filling is allowed to expand back into the apparatus as far as stopcock  $S_{14}$  (Fig. 63) with all stopcocks leading to the side arms ( $S_8$ ,  $S_9$ , and  $S_{13}$ ) open. After 10 min. it is condensed back into the counter and the background measured again. If the background is appreciably higher than before, the apparatus must be monitored further, section by section, until the source of activity is found. As already noted on page 132, care should be taken to avoid the introduction of grease into the line as this can be contaminated as easily as the grease on a stopcock or taper joint but is not so easy to remove.

The order of magnitude of the contamination occurring can be seen from the fact that none is observed after the passage through stopcocks of butane with a specific activity of the order of 1000 counts/min./ml. although butane of this activity will contaminate the stopcock above a side arm in which it is stored overnight.

#### WET COMBUSTION OF HIGH-SPECIFIC-ACTIVITY TRITIUM COMPOUNDS

The tritium-labeled compounds used in chemical and biochemical experiments must usually be assayed beforehand and always have a specific activity much too high for direct assay. As

an alternative to diluting with carrier it is convenient to assay substances of specific activity greater than about  $10^5$  counts/min./mg. by wet combustion.

*Procedure.* An amount of labeled material containing between 2 and 5 mg. carbon is weighed into a 20-ml. test tube fitted with a standard taper joint and a small air condenser. For every milligram of carbon in the sample, 0.5 ml. Van Slyke wet combustion fluid and 30 mg. potassium iodate are added and the mixture boiled for 2 to 3 min. All the hydrogen in the sample is thus converted into hydrogen ions and the solution can be diluted to a suitable volume with water. Thus if 2 mg. of a sample with a specific activity of  $10^7$  counts/min./mg. is combusted and the combustion fluid diluted to 100 ml., the solution will have a specific activity of 200 counts/min./mg. This is a convenient activity for assay, and 10 mg. of the solution can be converted to butane without danger of contaminating the apparatus.

#### REFERENCES

1. T. M. POWELL and E. B. REID, *J. Am. Chem. Soc.* **75**, 1020 (1945).
2. R. F. GLASCOCK, *Nucleonics* **9**, No. 5, 28 (1951).
3. R. L. CHASSON and M. L. MACKNIGHT, *Rev. Sci. Instr.* **24**, 212 (1953).

## CHAPTER X

### THE PREPARATION OF HEAVY NITROGEN SAMPLES FOR MASS SPECTROMETRIC ANALYSIS

Heavy nitrogen,  $N^{15}$  of natural abundance 0.38 atoms per cent, is usually analyzed in the mass spectrometer in the form of nitrogen gas. The determination of this isotope in any compound, therefore, involves conversion of combined nitrogen to elemental form. Two classical methods have been adapted to isotopic analysis. These are the manometric micro-Kjeldahl and the Dumas methods. In the micro-Kjeldahl method, the nitrogen in the compound is first converted to ammonia by digestion with concentrated sulphuric acid followed by decomposition of the ammonia to nitrogen with sodium hypobromite



In the Dumas method, the compound is oxidized by copper oxide in an atmosphere of carbon dioxide, the resulting oxides of nitrogen (chiefly  $N_2O$ ) reduced to nitrogen with hot copper, and the nitrogen collected over concentrated alkali which absorbs the carbon dioxide.

#### MICRO-KJELDAHL METHOD (RITTENBERG)

##### *Preparation of Ammonia*

Enough of the organic sample to yield 1 mg. nitrogen (about 0.8 ml.) is digested in a semimicro-Kjeldahl flask with a mixture consisting of 2 ml. concentrated sulphuric acid, 50 mg. of a 3-to-1 mixture of copper and potassium sulphates, and 1 granule of selenium. Digestion is carried out for 12 to 18 hours in order to oxidize all organic matter. Incomplete oxidation results in the production of volatile substances, such as methylamine and ethylamine, which escape oxidation by hypobromite and contaminate the nitrogen. They then give rise in the spectrometer



to ions of masses 29, 31, and 45. Since the ion peak 29 ( $N^{14}N^{15}$ ) is the one which is to be measured, the presence of these impurities will obviously introduce a serious error into the determination. Rittenberg (1) found that an additional difficulty arising from the presence of these impurities was their interference with the operation of the spectrometer and the long time taken to get rid of them, a thorough baking under high vacuum being necessary. He found that they arise particularly from lysine, creatine, sarcosine, arginine, and ornithine. He also found that not all amino acids yield ammonia smoothly by the usual oxidation procedure. Lysine in particular is completely oxidized only if mercuric sulphate is substituted for the copper and potassium sulphate mixture. Holt and Hopson-Hill (2) found that azobenzenes were particularly resistant to oxidation by this procedure, only about 7% of the expected yield of ammonia being obtained. This yield was increased to 70% if glucose was used as a catalyst instead of selenium.

After the digestion is complete the mixture is cooled, diluted with 25 ml. water, and 40% sodium hydroxide solution added until alkaline. The ammonia is then distilled off into 5 ml. of  $N/20$  hydrochloric acid. The apparatus recommended for this procedure is made entirely of glass, with standard taper joints, so that it can be thoroughly washed to prevent the contamination of one sample with another.

### *Oxidation of Ammonia to Nitrogen*

*Sodium hypobromite solution.* 200 gm. of sodium hydroxide are dissolved in 300 ml. water and divided into two portions. To one portion, 60 ml. bromine is added over a period of about 10 min. with continuous stirring until dissolved. The other portion of sodium hydroxide solution is then added and the solution stored in the refrigerator where it will keep its activity for several months. The precipitate of sodium bromide which forms during the first four days is filtered off. 1 ml. of the solution is equivalent to about 10 mg. ammonia.

*Apparatus.* A form of Rittenberg's apparatus is shown in Fig. 64. The reaction vessel A has two limbs and can be attached to a

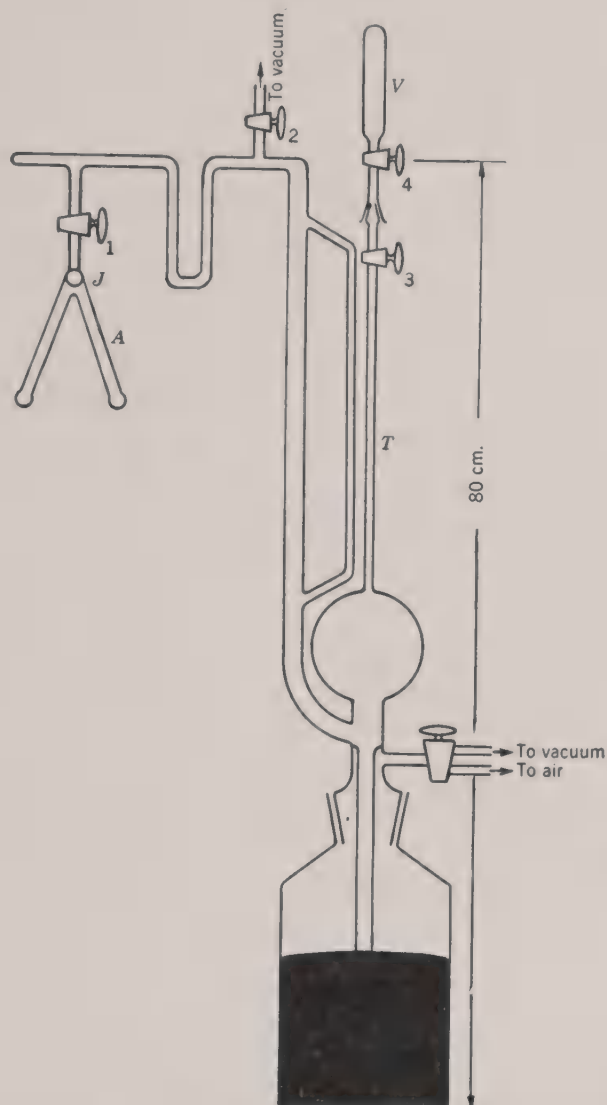


FIG. 64. Apparatus for collecting heavy nitrogen (micro-Kjeldahl). A: reaction vessel; J: standard taper joint with axis horizontal; T: Calibrated tube of Toepler pump; V: gas-sample tube; 1-4: Stopcocks. (Based on apparatus designed by D. Rittenberg.)

vacuum line by means of a standard joint *J*. This joint is horizontal so that *A* can be swiveled in a vertical plane. The gas sample vessel, which may be either a break-seal tube or a closed tube fitted with a stopcock as shown in the diagram, is attached to the delivery side of the Toepler pump.

*Procedure.* The ammonia solution is tested with indicator paper to make sure that it is acid and then concentrated to about 1.5 ml. It is transferred to one of the limbs of the reaction vessel *A* and an excess of sodium hypobromite solution, diluted with an equal volume of water, added to the other. The device is attached to the apparatus at *J*; both limbs are then chilled in liquid air or solid carbon dioxide and evacuated through stopcocks 1 and 2. Stopcock 1 is then closed, and after the two liquids have been warmed to room temperature they are mixed by tilting the reaction vessel. When the reaction subsides, both limbs of vessel *A* and the U-trap are chilled in liquid air or solid carbon dioxide and stopcock 1 opened, allowing the nitrogen in *A* to expand into the Toepler pump. Then, by raising the mercury in the pump, the gas is compressed into the previously evacuated sample tube *V*; stopcocks 3 and 4 are then closed and the mercury lowered again. If desired, the tube *T* can be calibrated as for a McLeod gauge (page 34) so that the volume of gas can be measured.

Precautions must be taken at all stages to avoid contamination of the sample by atmospheric air. Its presence is indicated by peaks at mass 32 and 40, corresponding to oxygen and argon.

#### MICRO-DUMAS METHOD (HOLT AND HUGHES)

Holt and Hughes (3) have modified the standard micro-Dumas apparatus so that the nitrogen collected in the nitrometer can be transferred to a break-seal tube. The technique was originally designed for the purpose of studying the thermal decomposition of  $N^{15}$ -labeled hydrazobenzene but it has been found suitable for use with such substances as potassium phthalimide, azobenzene, amino acids, and proteins.

#### *Apparatus*

*Combustion tube.* This is made of quartz and is 50 cm. long and 10 mm. in diameter. It is fitted with standard taper sockets

at each end. The *permanent filling*, starting from the end nearest the nitrometer, consists of 21 cm. of copper oxide made from wire, A, and 4 cm. of reduced copper, B, as shown in Fig. 65. The reduced copper is prepared by heating a 4-cm.-long zone of copper oxide in a stream of hydrogen. The *temporary filling*, which is renewed for each combustion, consists of 8 cm. of copper oxide made from wire, C, and 7 cm. of copper oxide powder, D, in the

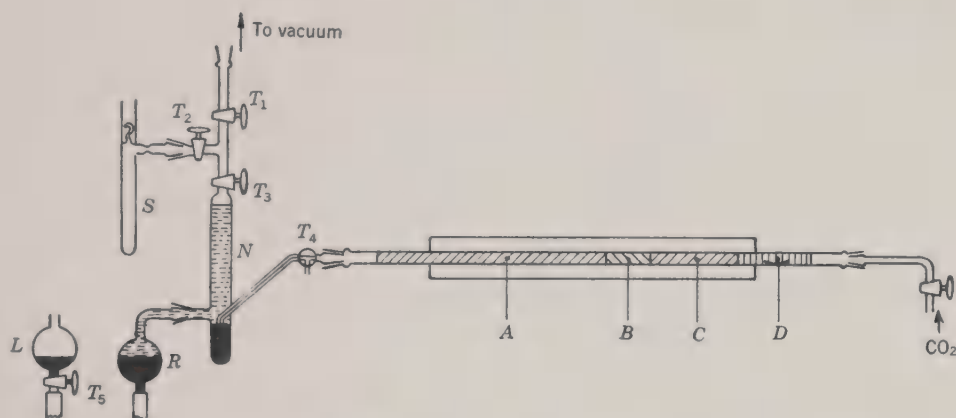


FIG. 65. Apparatus for collecting heavy nitrogen (Holt and Hughes).

A-D: Filling of combustion tube.

A: Copper oxide made from wire (21 cm.).

B: Reduced copper (4 cm.).

C: Copper oxide made from wire (8 cm.).

D: Copper oxide powder (7 cm.) covering combustion boat.

N: Nitrometer.

R: Mercury and alkali reservoir bulb.

L: Leveling bulb.

S: Break-seal sample tube.

T<sub>1</sub>-T<sub>5</sub>: Stopcocks.

middle of which is placed a combustion boat containing the sample.

With the exception of the 4 cm. nearest the nitrometer, the whole of the permanent filling and about 1 cm. of the temporary filling are maintained at 600° by means of an electric furnace 30 cm. long. The temporary filling and sample are heated to about the same temperature during each combustion with either an auxiliary furnace or a gas burner.

The elemental copper in the permanent filling serves to re-



duce any oxides of nitrogen formed. The 4 cm. of unheated copper oxide is to promote the recombination of any carbon monoxide and oxygen that may have resulted from decomposition of carbon dioxide. This is important because carbon monoxide gives mass peaks of 28 and 29 ( $C^{12}O$  and  $C^{13}O$ ), coinciding with those of the nitrogen peaks ( $N^{14}N^{14}$  and  $N^{14}N^{15}$ ).

*Nitrometer.* The nitrometer  $N$  is connected to the furnace by means of a delivery tube bearing the two-way stopcock  $T_4$ . This delivery tube dips below the surface of mercury in the nitrometer, forming a seal. A 40% solution of potassium hydroxide is contained in the nitrometer and reservoir bulb  $R$ ,  $R$  being connected by heavy rubber tubing to a leveling bulb,  $L$ , bearing a stopcock  $T_5$ . The nitrometer is evacuated through stopcocks  $T_3$  and  $T_1$  by pumps giving a vacuum of about  $10^{-6}$  mm. mercury pressure. A standard taper joint is attached to stopcock  $T_2$  for the purpose of connecting the break-seal gas sample tube.

### *Procedure*

The organic sample, weighing between 10 and 15 mg., is weighed into a porcelain boat, introduced into the combustion tube, and covered with copper oxide powder as already described. The combustion tube is swept clear of air by allowing carbon dioxide to pass through it overnight and run to waste through the side arm of stopcock  $T_4$ . Half an hour before a combustion is due to begin, the potassium hydroxide solution is outgassed by exposing it to the vacuum pumps. For this purpose the leveling bulb is lowered, with stopcock  $T_5$  open, until the solution half fills the reservoir bulb  $R$ . The leveling bulb is then clamped in position, with stopcock  $T_5$  closed, and stopcocks  $T_1$  and  $T_3$  are opened to the vacuum line, the pumps being protected from water vapor with a cold trap.

When the combustion is about to begin, stopcock  $T_3$  is closed, stopcock  $T_5$  opened, and the leveling bulb raised until the potassium hydroxide completely fills the nitrometer, and then clamped in position with  $T_5$  open. Care must be taken that no alkali gets into the bore of stopcock  $T_3$ , otherwise it is almost certain to leak. Stopcock  $T_4$  is adjusted to connect the combustion tube with the

nitrometer, and the carbon dioxide supply is turned off. Having made sure that the permanent filling is at the right temperature, combustion is then carried out by heating the sample and temporary filling to about 600°. Carbon dioxide is displaced into the nitrometer when heating starts and forms rapidly diminishing bubbles in the alkali which disappear before they reach the top. Later the nitrogen formed in the combustion tube reaches the nitrometer and collects above the surface of the alkali. When no more is produced, the gaseous contents of the combustion tube are displaced into the nitrometer with a stream of carbon dioxide and stopcock  $T_4$  then closed.

The sample tube is attached to its socket and evacuated through stopcocks  $T_2$  and  $T_1$ . These stopcocks are then closed and  $T_3$  carefully opened, allowing the nitrogen in the nitrometer to expand into the space between  $T_1$ ,  $T_2$ , and  $T_3$ .  $T_2$  is then opened, allowing the gas to enter the sample tube. Residual gas in the nitrometer can be transferred to the sample tube if necessary by repeating this process using the leveling bulb and nitrometer contents as a Toepler pump. Finally, the sample tube is sealed off and withdrawn from the apparatus.

#### REFERENCES

1. D. RITTENBERG, in "Preparation and Measurement of Isotopic Tracers" (D. W. Wilson, A. O. C. Nier, and S. P. Reimann, eds.) p. 31. J. W. Edwards, Ann Arbor, Michigan, 1946.
2. P. F. HOLT and B. I. HOPSON-HILL, *J. Chem. Soc.* **1952**, 4251.
3. P. F. HOLT and B. P. HUGHES, *J. Chem. Soc.* **1953**, 1666; also private communication.

## CHAPTER XI

### THE PREPARATION OF TRITIUM-LABELED COMPOUNDS

Tritium is particularly useful for the preparation of labeled compounds which are required in extremely high specific activities, which are very difficult to synthesize from  $C^{14}$ , or which, when labeled with  $C^{14}$ , are prohibitively expensive in the activities required. Since, however, all the syntheses described in the vast literature on deuterium-labeled compounds are equally applicable to tritium, it is clearly impossible to give an account of more than a small proportion of them. A selection of the more important synthetic methods has therefore been made, with special reference to those which are of general application; and although the preparation of specific compounds is described, it is hoped that it will not be difficult, on the information given, to extend at least one of the techniques to the preparation of any other labeled compound that may be required.

The methods most commonly used for the introduction of isotopic hydrogen into organic compounds depend on the hydrogenation of double bonds or on the exchange—usually metal-catalyzed—between a normal specimen of the substance to be labeled and a relatively simple substance, such as water, sulphuric acid, or acetic acid, containing a high abundance of the isotope. Tritiated water and acetic acid may be available commercially but, if not, their preparation from tritium gas will be necessary. Tritiated sulphuric acid is prepared by dissolving the calculated amount of sulphur trioxide in tritiated water.

#### GENERAL PRECAUTIONS

The health hazard arising from tritium is not very great owing to the very low energy of its beta particles, the high dilution which it undergoes once metabolized to water in the animal

body, and the rapid turnover which then follows. Reasonable precautions should nevertheless be taken against inhalation or ingestion of tritium or its compounds. This means a well-ventilated laboratory, the vent tubes of the vacuum pumps being led outside the building, and particular care being taken not to break apparatus containing high activities. Great care should also be taken not to contaminate the apparatus used for assay. Under no circumstances should high activities of tritium be manipulated in apparatus which will ever be connected into that used for the assay of either  $C^{14}$  or tritium. Some vacuum apparatus is unavoidable for the synthesis of tritium compounds and it should be provided with entirely separate pumps. Tritium-hydrogen dissolves in stopcock grease which will therefore be a constant source of contamination to counters and, if a permanently elevated background is to be avoided, they should never be attached to the synthesis apparatus.

#### THE PREPARATION OF TRITIATED WATER

Tritium-hydrogen can be oxidized by a number of procedures of which the commonest are passage over hot copper oxide or explosion with oxygen. With care these methods can be applied quite safely, although there is always an inherent risk of a shattering explosion. The most satisfactory method, and one which will deal with quantities of the order of 100 standard ml. hydrogen in an hour, is that used at the British Atomic Energy Research Establishment and described by Wilson (1).

##### *Catalytic Palladium Diffusion Method*

In this method, advantage is taken of the fact that hot palladium is permeable to hydrogen and its isotopes. Tritium-hydrogen is allowed to diffuse through a heated palladium tube contained in a chamber of oxygen. Catalytic oxidation takes place on the surface of the palladium and the water formed is condensed in a tube for removal. One of the advantages of the method is that impurities in the tritium cannot pass through the palladium and interfere with the reaction or contaminate the water because palladium is not permeable to them.



The apparatus used is shown in Fig. 66. The oxidizer *L* consists of a thin palladium tube 3.8 cm. long and with an internal diameter of 1.6 mm. It is attached to the Pyrex glass parts through short platinum tubes of the same diameter, to which it is gold

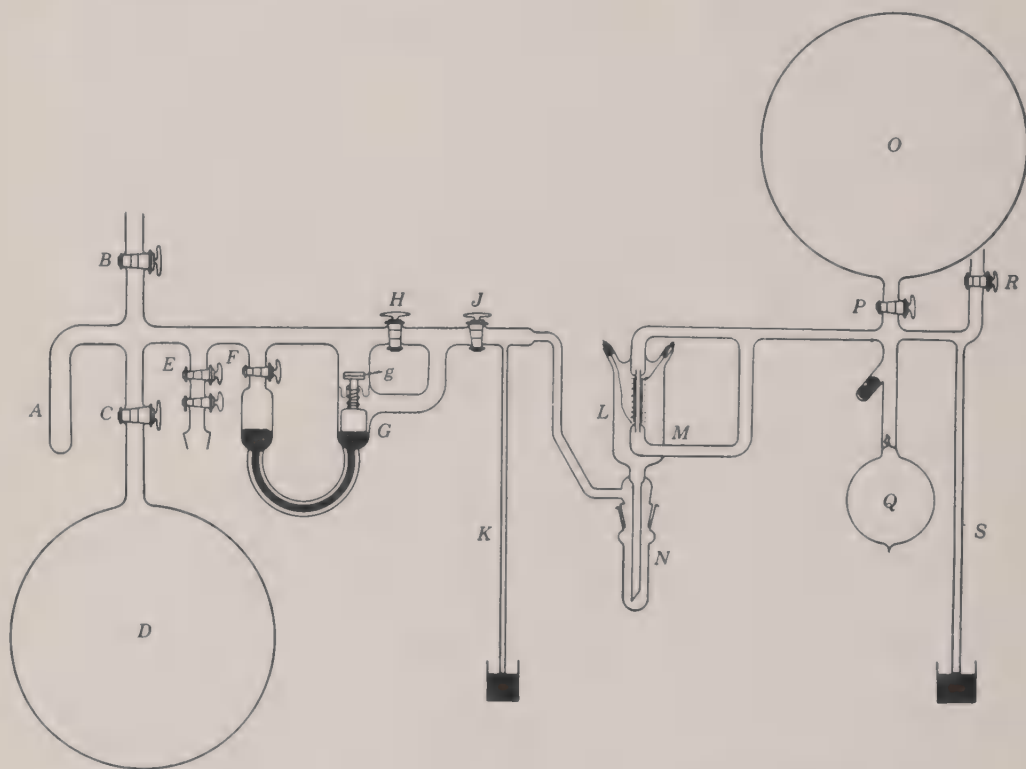


FIG. 66. Apparatus for the preparation of tritiated water (Wilson).

A: Cold finger for liquefying oxygen with liquid nitrogen.  
 B, C, E, F, H, J, P, R: Stopcocks.  
 D: Bulb of oxygen.  
 G: Float of reducing valve.  
 g: Gasket.

K and S: Manometers.  
 L: Oxidizer.  
 M: Convection tube.  
 N: Collection tube.  
 O: Bulb of inactive hydrogen.  
 Q: Bulb of tritium.

(Diagram: British Crown Copyright. Published by permission of The Director, Atomic Energy Research Establishment, Harwell, England.)

soldered, and through lead glass and graded seals. The palladium tube is encased in a small silica tube wound with a platinum-ribbon heating element connected to tungsten-glass seals. The tritium is stored in bulb *Q* which is fitted with a break-seal. A

supply of pure hydrogen is stored in bulb *O*. This hydrogen is available to increase the volume of tritium when small samples of high specific activity are to be oxidized and the use of carrier is permissible.

Although there is every reason to believe that oxidation will take place quietly and safely with the oxygen at atmospheric pressure, the pressure of oxygen is nevertheless maintained below 20 cm. to eliminate completely the chance of a shattering explosion. The pressure is controlled by means of the glass reducing valve (designed by W. J. Arrol). It consists of a U-tube containing mercury and a stainless steel float *G*. The stem of the float passes through a glass tube whose upper end is ground to form a seating for the rubber gasket *g* which is held against it by the combined weight of the float and the pressure of gas above it. Oxygen from the reservoir *D* is condensed into the tube *A*, by cooling it to  $-196^{\circ}$  (liquid nitrogen) at which temperature oxygen has a vapor pressure of 15 cm. Stopcock *F* is then closed, thus trapping gas at this pressure in the space beneath it. Stopcock *H* is closed, *J* is opened, and the condensed oxygen in *A* allowed to warm to laboratory temperature. Now, as oxygen is consumed in the oxidizer *L*, the mercury in the right-hand limb of the reducing valve rises, because of the pressure of gas in the space beneath stopcock *F*, thus lifting the float and permitting gas to pass the valve into the apparatus to the right of *H*. When the pressure there is restored to 15 cm., the mercury levels stand at the same height and the valve closes.

Oxidation of tritium is carried out by first filling the oxidizer with oxygen at 15 cm. and then opening the bulb *Q* at the break seal. Current, under the control of a variable transformer, is passed into the heating element around the palladium tube until, at a temperature of about  $200^{\circ}$ , a falling pressure on the manometer *S* indicates that gas is beginning to diffuse through the palladium. Although impurities cannot pass through it, their accumulation in the palladium tube is prevented by the convection tube *M* which promotes circulation. The envelope of the oxidizer becomes quite warm during the run, and therefore the water formed condenses in the cooler tube *N*.

If only small quantities of tritium are to be oxidized, a more conveniently manipulatable amount of water may be obtained by diluting the tritium with carrier hydrogen from the reservoir O. Wilson recommends using sufficient hydrogen to produce about 0.5 ml. water. If the water were required carrier-free, however, it should be fairly simple to transfer it by a vacuum sublimation into a break-seal tube attached to a convenient point on the apparatus (not shown in the diagram), or to transfer it to an adjacent piece of apparatus. Wilson points out, however, that water in which 20% of the hydrogen has been replaced by tritium decomposes by radiolysis into hydrogen and oxygen, and pressures of up to 60 cm. have been built up within the space of about three months.

#### *Oxidation of Tritium-Hydrogen by Explosion*

Small quantities of tritium-hydrogen may be conveniently converted to water by a nonshattering explosion provided the pressure at which it takes place does not exceed 20 cm. Before tritiated water became generally available, the manipulation and combustion of tritium was carried out in the author's laboratory by means of the apparatus shown in Fig. 67. This apparatus was not specifically designed for this purpose but it is actually the microhydrogenation apparatus whose use is described later (see Fig. 71). *Tr* is a Toepler pump and *Z* is a combustion bulb of a size depending on the volume of gas to be combusted. For example, a 500-ml. bulb will contain about 90 standard ml. of hydrogen when filled with chemically equivalent quantities of hydrogen and oxygen at a total pressure of 20 cm. *Z* is fitted with two electrodes whose internal extremities are about 2 cm. apart. One of them is connected to ground and the other to the high-frequency coil arranged so that it can be switched on from a safe distance. A spark can thus be made to pass between the electrodes. The tritium is contained in the bulb *Tr*. Oxygen, purified on the way by passage through a heated tube of platinized asbestos followed by an anhydrone-carbosorb tube, is passed into the device beneath stopcock *S*<sub>63</sub> and runs to waste at the jet. Hydrogen, purified by passage through a U-tube of granular

charcoal cooled in liquid nitrogen is burned at the jet beneath stopcock  $S_{64}$ .

For the preparation of low-specific-activity water, the following preparation actually carried out in the author's laboratory may be taken as an example: 50 ml. tritium-hydrogen believed

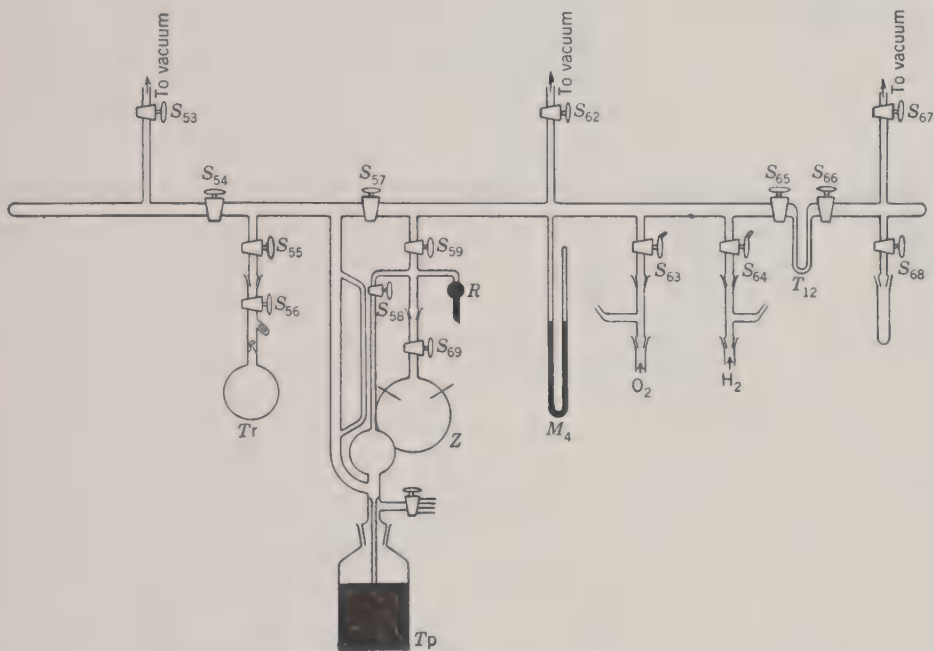


FIG. 67. Permanent high-vacuum line apparatus for combustion of tritium hydrogen by explosion.

$M_4$ : Manometer.

$S_{53}$ – $S_{69}$ : Stopcocks.

$T_{12}$ : U-trap.

$T_r$ : Bulb of tritium.

$T_p$ : Toepler pump.

$R$ : Mercury bulb. For use, see Fig. 71 and text.

$Z$ : Explosion bulb.

to contain 500 mc. of activity was obtained sealed up in a 250-ml. bulb. This was sealed to a stopcock fitted with a side arm, to hold the steel weight by means of which the break-seal is opened, and a standard socket. It was attached to the apparatus, the space above the break-seal evacuated, stopcock  $S_{55}$  closed, and the seal opened. No measurement of the gas in the Toepler pump was possible because the volume (50 ml.) was too great. The volume stated on the package was therefore assumed to be correct and



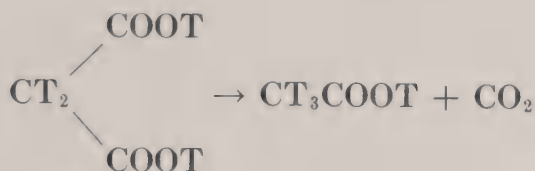
the gas was pumped directly into the previously evacuated bulb Z, whose volume was known to be 500 ml., and stopcock  $S_{59}$  closed. The pressure of tritium-hydrogen was therefore now 0.1 atmosphere or 7.6 cm. The vacuum line to the right of stopcock  $S_{57}$  was then filled with inactive hydrogen to a pressure of 12 cm. (registered on  $M_4$ ) and stopcock  $S_{59}$  opened. The pressure, which immediately fell, was quickly restored to 12 cm. by operating stopcock  $S_{64}$ , and stopcock  $S_{59}$  was then closed. It is to be noted that the addition of inactive hydrogen in this way sweeps tritium from the dead space below stopcock  $S_{59}$  into the combustion bulb. The line to the right of  $S_{57}$  was evacuated and then filled with oxygen at a pressure which a previous experiment had shown would fall to 20 cm. when expanded into the bulb of hydrogen. Stopcock  $S_{59}$  was opened and closed rapidly, the pressure on the manometer checked to make sure the pressure in the bulb was not more than 20 cm., and, as already described, the high-frequency coil switched on from a safe distance. Combustion immediately occurred, accompanied by a flash and a high-pitched pop. The radioactive water thus formed was stripped from the excess of oxygen by pumping the contents of the bulb through the U-trap  $T_{12}$ , cooled in liquid nitrogen, and was then transferred into a tube attached beneath stopcock  $S_{63}$ , sealed off, and removed from the apparatus.

#### PREPARATION OF TRITIUM-LABELED ACETIC ACID

Although the labeling of acetic acid with hydrogen isotopes by metal-catalyzed exchange at elevated temperatures has been described, the most efficient and convenient method is that of Halford and Anderson (2). This method makes use of the fact that when malonic acid is dissolved in water complete exchange of all the hydrogen atoms takes place. Thus in tritiated water of a given specific activity, the malonic acid becomes labeled to a specific activity depending on the relative proportions of acid and water. It follows from molecular weights that if malonic acid is dissolved in an equal weight of water, the proportions will be 6 moles of water to 1 mole of acid, and the specific activity of the hydrogen of both substances will be 75% of the original spe-

cific activity of the water. A higher ratio of water to acid will give a product with a higher specific activity but this is seldom necessary.

On decarboxylation of the malonic acid, completely labeled acetic acid is obtained:



The labile tritium of the acetic acid may be removed by conversion to the salt and subsequent regeneration of the acid.

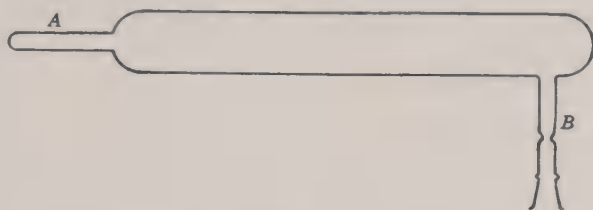


FIG. 68. Apparatus for the decarboxylation of malonic acid. The malonic acid is placed in tube A, and the vessel evacuated through B and sealed at the constriction. On heating the malonic acid it decomposes and acetic acid collects in B.

Whereas the quantities of deuterium-labeled acetic acid usually required are large enough for the application of conventional-scale synthetic methods, it may be necessary to work on a much smaller scale with tritium. In the author's laboratory the following method is used:

From 0.2 to 0.5 gm. pure malonic acid is dissolved in an equal weight of tritiated water, and the water then recovered by sublimation *in vacuo*. The dry malonic acid is then transferred to the side arm A attached to the Pyrex glass vessel shown in Fig. 68. The vessel is large enough to accommodate all the carbon dioxide evolved in decarboxylation. The vessel is evacuated through the tube B and sealed off at the constriction. When the seal is cool the vessel is inserted as far as possible into a tubular heating oven

of the type used for baking counters, and *B* is cooled in a solid carbon dioxide bath. The temperature of the oven is raised to 190°–200° and maintained there for 15 min. During this time, glacial acetic acid collects in the cooled side arm *B*, which is cut off and weighed. After removal of the acetic acid it is weighed again. The yield is usually about 85%. If the acetic acid is required in the form of sodium acetate, it is dissolved in a few milliliters of water and titrated with alkali previously standardized against a weighed amount of authentic acetic acid. It has been found that the labeled material has exactly the same equivalent weight. The sodium acetate can be recovered in the solid anhydrous state by freeze-drying on the vacuum system.

### The Preparation of Tritiated Organic Compounds by Exchange WITHOUT CATALYST

#### *Alpha-labeled Fatty Acids*

Although the exchange between carbon-bound hydrogen of the substance to be labeled and an isotopic solvent is usually promoted with metal catalysts, they are not always necessary. Thus van Heyningen, Rittenberg, and Schoenheimer (3), making use of a reaction discovered by Ingold, Raisin, and Wilson (4), have prepared deuterio-palmitic acid by heating normal palmitic acid with deuterio-sulphuric acid containing 10%  $D_2O$  at 98°–100° for 50 hours. The product contained 4.32 atoms per cent excess deuterium, which corresponded to about 1.5 atoms deuterium per molecule palmitic acid. On bromination, almost exactly half the deuterium was lost, showing that nearly all of it was attached to the alpha-carbon atom. Although this deuterium content was barely high enough for biological experiments, the method could obviously be usefully extended to tritium labeling because of the high activities of tritium available and the sensitivity of the methods used for detecting it. Indeed, high tritium activities would make unnecessary the preparation of tritio-sulphuric acid since all the isotope could be supplied in the form of added water. The instructions of van Heyningen and co-workers would then be modified as follows:

After 1.85 gm. of 100% sulphuric acid and 0.185 ml. tritiated water are mixed in a test tube, 1 gm. palmitic acid is added. The tube is then sealed off and heated in an oven at  $98^{\circ}$ – $100^{\circ}$  for 50 hours. At the end of this time the mixture is dark amber in color. The tube is opened and the solution diluted with water and extracted with ether. The ethereal solution is extracted with alcoholic potassium hydroxide solution, to displace the labile hydrogen of the carboxyl group, and the alkaline solution acidified with dilute sulphuric acid and extracted with ether. The ethereal solution is thoroughly dried, the ether evaporated off, and the acid recrystallized from aqueous acetone. The yield is about 0.9 gm. and the specific activity depends on that of the water originally used. If this were, for example, 100 mc./ml., then it may be calculated from the data given by van Heyningen and co-workers that the specific activity of the palmitic acid would be 1.7 mc./gm. This could be made three times as high if necessary by using tritio-sulphuric acid since the quantities used correspond to 2 moles sulphuric acid to 1 mole water, causing a threefold dilution of the tritium.

#### USING METAL CATALYSTS

Because much more isotope can be introduced into organic molecules by means of metal-catalyzed reactions, these are the ones most commonly used, and the catalyst usually recommended is Adams' platinum oxide catalyst. This is usually represented at  $\text{PtO}_2$  and is reduced to metallic platinum with isotopic hydrogen gas. A convenient method of preparation is that described by Vogel (5) and is as follows:

##### *Adams' Platinum Oxide Catalyst*

First, 3.5 gm. of pure chloroplatinic acid is weighed into a 250-ml. beaker and 10 ml. water added. Then when the chloroplatinic acid is dissolved, 35 gm. of pure analytical grade sodium nitrate is added and the mixture evaporated to dryness over a burner, with constant stirring. Heating is continued until the temperature reaches  $350^{\circ}$ – $370^{\circ}$  when fusion occurs. Brown fumes are evolved and a precipitate of platinum oxide gradually sepa-



rates. Frothing-over may be checked by directing the flame of another bunsen burner at the top of the reaction mixture; the burner beneath the beaker should not be removed in case the top of the melt solidifies. Heating is continued until the temperature reaches  $550^{\circ}$  when gas evolution will nearly have ceased. The melt is maintained at this temperature for 30 min. and then allowed to cool. Then 50 ml. water is added to the solid product and a brown precipitate of platinum oxide (said to be  $\text{PtO}_2 \cdot \text{H}_2\text{O}$ ) settles out. It is thoroughly washed and dried in the oven. It reacts with hydrogen at room temperature and is usually reduced in the flask in which the exchange is to take place.

### *Uniformly-labeled Fatty Acids*

Van Heyningen, Rittenberg, and Schoenheimer (3) use catalytic exchange for the transfer of deuterium from heavy water to a variety of long-chain fatty acids and achieve up to 85 or 90% exchange. The deuterium is not, however, attached to any particular carbon atom but is believed to be uniformly distributed along the carbon chain. For tritium labeling, therefore, unless very high specific activities are required, the method may be less useful than the sulphuric acid exchange method described above because the product is not specifically labeled. These workers based their method on the finding of Horrex and Polanyi (6) that hydrocarbons such as isopentane and cyclohexane become labeled with deuterium if heated with heavy water and platinum for several days at  $120^{\circ}$ . Only very little deuterium is introduced into fatty acids under these conditions, however, unless a small quantity of alkali is added. The amount required to promote exchange is considerably less than that required to neutralize the acid, and van Heyningen, Rittenberg, and Schoenheimer suggest that this is because exchange takes place only between water and dissolved soap which in turn is in equilibrium with undissolved fatty acid. Basic catalysis may also be operating.

*Procedure.* 750 mg. platinum oxide and 7.5 ml. isotopic water are introduced into a narrow-neck, round-bottom flask. The catalyst is reduced by shaking with hydrogen gas at room temperature and atmospheric pressure until no further pressure

change occurs (750 mg.  $\text{PtO}_2 \cdot \text{H}_2\text{O} = 137 \text{ ml. H}_2$ ). Since randomization of hydrogen between gas and water occurs under the influence of the catalyst, deuterium gas should be used for the reduction when deuterium-labeled acids are to be prepared. It is not so necessary to take the corresponding precaution with tritium since some loss of activity from the water into the gas can usually be tolerated.

Next 7.5 gm. fatty acid and 100 mg. potassium hydroxide are added. The flask is cooled in a solid carbon dioxide bath, evacuated, sealed, and then shaken for six days while being maintained at a temperature of  $130^\circ$ – $135^\circ$ . At the end of this time it is opened and the water removed by sublimation *in vacuo*. The residue is acidified by adding phosphorus pentoxide, and the fatty acid extracted with ether. Since the labile hydrogen of the  $-\text{COOH}$  group will have become labeled, it is usually necessary to replace it with ordinary hydrogen. This is done by extracting the ethereal solution with dilute alkali, acidifying, and re-extracting with ether. The solution is dried, the solvent removed, and the acid purified by recrystallization. When van Heyningen, Rittenberg, and Schoenheimer carried out this preparation with lauric acid, they obtained a 96% yield, the product melting at  $45.2^\circ$  and containing 28.7 atoms per cent excess deuterium. This corresponds to 58% of complete exchange. They point out that the degree of exchange depends both on the amount of catalyst used and the time of treatment. Time is particularly important since equilibration is not complete even after 12 days. They found that the deuterium was stably bound, none being removed from deuterio-palmitic acid (5.49 atoms per cent excess) when refluxed for 48 hours with 20 ml. ethyl alcohol, 18 ml. water, and 4 ml. concentrated sulphuric acid.

### *Tritium-labeled Steroids*

The preparation of a deuterium-labeled steroid was first reported by Bloch and Rittenberg in 1943 (7). They obtained deuterio-cholesterol in 44% yield by means of a platinum-catalyzed exchange reaction between ordinary cholesterol and a 70% acetic acid-deuterium oxide mixture. They found that the higher the

deuterium content of the cholesterol isolated from the reaction mixture the poorer the yield, indicating that conditions which favor exchange also favor destruction of the sterol. By converting the cholesterol to cholesteryl chloride and submitting this compound to thermal degradation they were able to demonstrate that the isotope was distributed nearly equally between nucleus and side chain. The reaction was studied somewhat more closely in the following year by Anker and Bloch (8) who showed that the products formed when cholesterol is heated in diluted acetic acid solution with platinum consist chiefly of 4,5-cholestenone, cholestanone, coprostanone, cholestane, and coprostanone, together with unchanged cholesterol. The exchange reactions and distribution of the isotope have now been studied much more closely by Fukushima and Gallagher (9) and extended by them (10) as a preparative method for other labeled steroids.

*Tritium-labeled cholesterol.* The method used by Fukushima and Gallagher (9) follows that of Bloch and Rittenberg (7) and of Anker and Bloch (8). 1.25 gm. Adams' platinum oxide catalyst is suspended in a mixture of 40 ml. tritiated acetic acid ( $\text{CT}_3\text{COOH}$ ) and 13 ml. tritiated water of about the same isotopic abundance. The catalyst is reduced with tritium-hydrogen by shaking at room temperature, and 15.5 gm. pure cholesterol is then added. The mixture is frozen in a solid carbon dioxide bath and the vessel evacuated and sealed. The reaction mixture is shaken for three days while being maintained at a temperature of  $127^\circ \pm 2^\circ$ , the reaction vessel then opened, and the solvent removed *in vacuo*. The product consists of a partially acetylated mixture of the compounds listed in the preceding paragraph. It is hydrolyzed by refluxing for 1 hour with 200 ml. of 2.5% methanolic potassium hydroxide containing 10 ml. benzene. On chilling the solution, crystals separate and are filtered off. In an actual experiment using deuterium Fukushima and Gallagher obtained 13.6 gm. of crystals which, on recrystallization from acetone, ultimately yielded a total of 5.4 gm. deuterio-cholesterol, m.p.  $145.5^\circ$ – $147.5^\circ$ . The product was further purified by acetylating with acetic anhydride in pyridine at laboratory temperature, yielding, after recrystallization from ethanol, 5.25 gm. deuterio-cholesterol acetate, m.p.  $114.5^\circ$ – $116.5^\circ$  (corr.), con-



taining 5.15 atoms per cent excess deuterium or 2.48 atoms deuterium per molecule. This isotopic abundance is to be related, for the purposes of calculating the expected specific activity if tritium were used, to a deuterium abundance of 99.9% in the water, hydrogen, and acetic acid used in the reaction.

*Tritium-labeled progesterone.* 100 mg. Adams' platinum oxide catalyst are suspended in 2.4 ml. tritiated acetic acid made to 70% concentration with tritiated water and contained in a round-bottom flask. The platinum oxide is reduced with hydrogen at laboratory temperature and atmospheric pressure. When no more hydrogen is taken up it is replaced by nitrogen. 800 mg. progesterone in another 2.4 ml. of 70% acetic acid is added, the solution is frozen in a solid carbon dioxide bath, and the flask evacuated and sealed. It is shaken or rotated in an oven at 130° for two days. At the end of that time the flask is cooled and opened. The catalyst is filtered off and the solvent removed by distilling off under reduced pressure.

Labile hydrogen atoms are displaced by dissolving the crude product in 100 ml. of 2% methanolic potassium hydroxide and refluxing under nitrogen for 30 min. The reaction mixture is then worked up. In an actual experiment, Fukushima and Gallagher extracted 795 mg. yellow oil from the methanolic potassium hydroxide. The oil crystallized on standing. When purified chromatographically over alumina, it yielded progesterone of melting point 120°–121°,  $[\alpha]_D^{31} = +187^\circ$  in ethanol, and  $\epsilon_{2400} = 17,400$ . The isotopic acid-water mixture used in the preparation had been 99.5 atoms per cent excess deuterium, and the product contained 1.9 atom per cent excess deuterium or 0.47 atom deuterium per molecule of progesterone. From later eluates of the alumina, 17 $\alpha$ -progesterone of melting point 140°–142° was obtained.

*Other compounds.* Substantially the same technique has been applied by Fukushima and Gallagher to the preparation of other deuterium- or tritium-labeled steroids, including dehydroisoandrosterone, oestrone, and  $\Delta^4$ -androstene 3,17-dione. From this compound labeled testosterone can be prepared by ordinary chemical methods.

Bell and Thomson (11) have applied essentially the same methods to the preparation of simple aliphatic compounds labeled



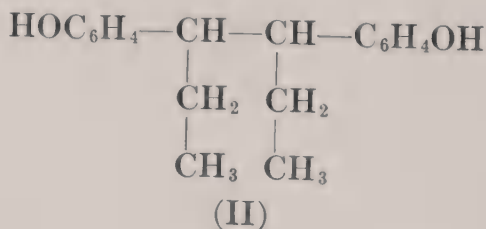
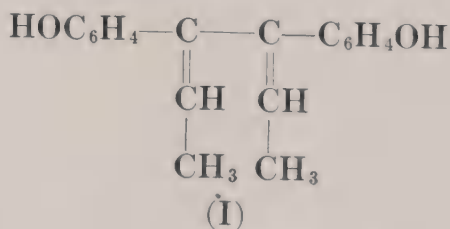
with deuterium. The compounds listed by them include hexane and cyclohexanol.

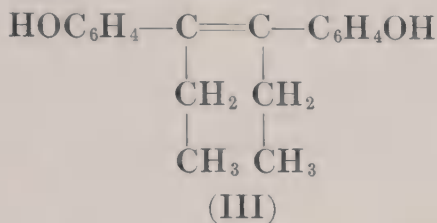
### Catalytic Hydrogenation

The introduction of hydrogen isotopes into organic compounds by the catalytic hydrogenation of double bonds affords a means of producing labeled compounds in which the position of the isotope is known with fair certainty. In some instances, however, its position needs to be confirmed since, according to Fukushima and Gallagher, when steroids are treated in this way the isotope does not necessarily become attached to the carbon atoms which have been reduced. When the desired unsaturated product is available, this method is probably easier to apply than the exchange method.

#### *Tritium-labeled Hexoestrol*

Williams and Ronzio (12) apply an ordinary-scale hydrogenation technique to the preparation of tritium-labeled hexoestrol (II) from dienoestrol (I). Dienoestrol was chosen rather than stilboestrol (III) because it is known from the work of Campbell, Dodds, and Lawson (13) that the hydrogenation of stilboestrol yields a mixture of *meso*- and *isohexoestrol* of which only the *meso*- form is physiologically active. The hydrogenation of dienoestrol, however, with a suitable catalyst yields a product consisting predominantly of *mesohexoestrol*.





Williams and Ronzio carry out the tritiation in an apparatus illustrated in Fig. 69. With each run, most of the apparatus is sealed off, detached, and subsequently destroyed, thus reducing any possible health hazard from contaminated glass.

*Procedure.* Tritium diluted with hydrogen is sealed in the U-tube *A* beneath the two break-seals *Y* and *Z*. The reaction is carried out in the flat-bottom flask *B* provided with a magnetic stirrer *S*. Into this flask is placed 500 mg. dienoestrol, 50 mg. 10% palladium-on-charcoal, and 5 ml. acetone. The flask is then chilled in liquid nitrogen and the entire apparatus evacuated to a pressure of about  $5 \times 10^{-3}$  mm. It is then removed from the pumping system by sealing off at the constrictions *F* and *G*.

The contents of *B* are allowed to warm up and the break-seal *Y* opened, thus allowing the tritium to expand into the reaction flask. It is allowed to react for 45 min., the mixture being constantly stirred. During this period the gas burette *D* is filled with inactive hydrogen from a cylinder. The stirrer is stopped and *K* raised so that a fairly high pressure obtains in *D*, and the break seal *Z* is broken. The unreacted tritium remaining in *A* and the intervening dead space is thus swept into the flask. The reading on the gas burette is taken and stirring resumed. The progress of the reaction can now be followed by taking frequent readings on the gas burette. It is complete in about 40 min. The mercury in the burette is then lowered to reduce the pressure in the system as far as possible, and the solvent in *B* is distilled off into *C*. The constriction *H* is then sealed and *B* removed.

The crude product is partly purified by redissolving in acetone and filtering through a bed of diatomaceous earth and decolorizing charcoal. The yield of product then obtained was found in all experiments by the authors to be more than 100% and was sometimes as high as 123% irrespective of whether the reaction was

carried out in acetone or ethanol. The excess weight is attributed to solvent of crystallization. They found, further, that the product consisted of a mixture of *meso*- and *isohexoestrol* which they separated by crystallization from benzene. From the published solubility data for *meso*-hexoestrol in benzene they calculated

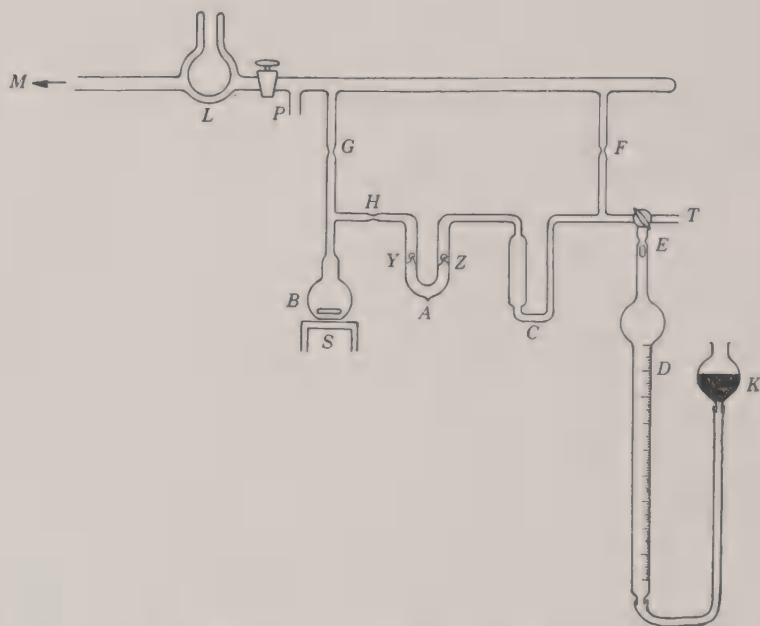


FIG. 69. Apparatus for the preparation of tritio-hexoestrol (Williams and Ronzio).

A: U-tube of tritium.  
 B: Hydrogenation vessel.  
 C: U-trap.  
 D: Gas burette.  
 E: Valve to prevent mercury from flooding into apparatus.

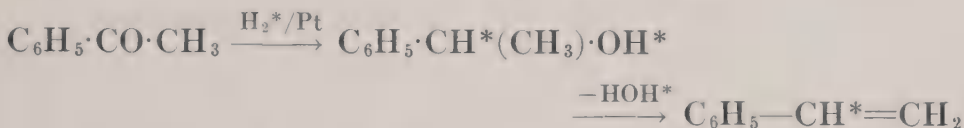
H, F, and G: Constrictions for sealing off.  
 K: Mercury reservoir.  
 L: Liquid-air back-diffusion trap.  
 M: To pumps.  
 P: To Pirani gauge.

that recrystallization from a solution prepared by dissolving 100 mg. hexoestrol in 5 ml. benzene should yield a 90% recovery. By applying this technique and bulking the several crops of crystals obtained after concentrating mother liquors they obtained a total yield of 72–77%, the product melting at 184°–185°. When 500 mc. tritium made to a volume of 5 ml. with inactive hydrogen was used, a product with a specific activity of about 1 mc./mg.

was obtained. The authors also record the interesting observation that the reaction proceeded significantly more quickly in the presence of this level of radioactivity than when only inactive hydrogen was used. They believe this to be due to some phenomenon such as activation of reactants.

### *Tritium-labeled Styrene*

A method for the reduction of the carbon-oxygen double bond in acetophenone with tritium-hydrogen has been described by Berstein, Bennett and Fields (14). This reaction yields methylphenylcarbinol which can be dehydrated to styrene:



These workers claim that their apparatus, illustrated in Fig. 70, is simpler and more convenient than that of Williams and Ronzio; certainly the necessity of sealing the apparatus and detaching from the vacuum system is avoided. As in the method of Williams and Ronzio, however, a large amount of inactive carrier hydrogen is used and consequently very high specific activities are not attainable.

*Procedure.* 5 gm. redistilled acetophenone and 300 mg. Adams' platinum oxide catalyst are placed in a hydrogenation bottle which is then connected to the apparatus and clamped in the shaker. As much air as possible is withdrawn from the system with a water aspirator and the apparatus flushed several times with inactive hydrogen admitted through stopcock *G* from a cylinder. The mercury in the gas burette is raised to the top and the pressure in the apparatus reduced to about 15 mm., again with the water aspirator. Stopcocks *C* and *F* are closed and the tritium ampoule opened at the break seal. The mercury in the burette is lowered, thus withdrawing most of the tritium from the ampoule, and stopcock *D* closed. The ampoule is then filled with inactive hydrogen to 1 atmosphere pressure, stopcock *F* closed again, and the hydrogen in the ampoule again withdrawn into the burette. Mixing is ensured by repeatedly raising and lowering the mercury



with stopcocks *D* and *E* open. This process is repeated until there is about 150 ml. gas in the burette. The shaker is then started and the tritium-hydrogen transferred to the hydrogenation bottle by opening the intervening stopcocks and raising the mercury in the burette. When this quantity of gas has been consumed, another 150 ml. is taken in the same manner and transferred to the hydrogenation bottle. The procedure is repeated four or five times, thus ensuring the transfer of all the tritium from the ampoule to the

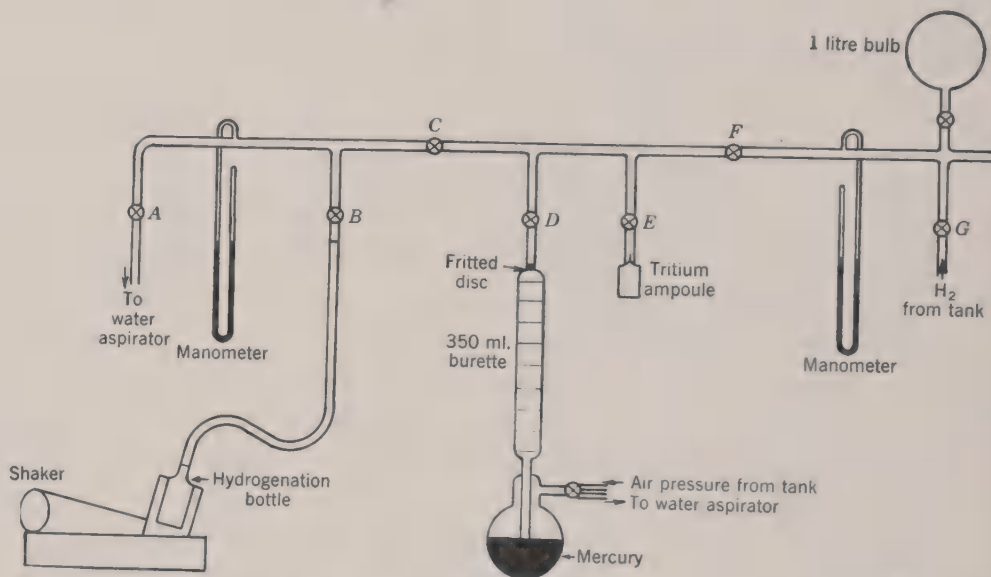


FIG. 70. Apparatus for the preparation of tritio-styrene (Berstein, Bennett, and Fields). A-G: Stopcocks.

hydrogenation bottle, or until no more gas is taken up (5 gm. acetophenone = 910 std. ml. hydrogen). The time required for the whole procedure is 3–4 hours. At the end of this time the product (tritiated methylphenylcarbinol) is isolated and purified by conventional chemical procedures or converted directly to styrene. For this purpose the recommended procedure is to add 10 mg. picric acid and 5 mg. *p*-toluenesulphonic acid to the crude carbinol in a flask and 10 mg. picric acid to the receiver. The reaction mixture is then distilled under reduced pressure using an oil bath at 155°–190° when water and styrene distill off (42°–68°

at 100 mm.). The distillate is dried over anhydrous magnesium sulphate. In an actual experiment, the authors obtained styrene (boiling point  $62.5^{\circ}$ – $63.5^{\circ}$  at 60 mm.) in 79% yield calculated on the original acetophenone used. The specific activity was 0.66 mc./mM.

It is interesting to note that when the product was oxidized to benzoic acid not all the activity was lost; about 9% remained in the benzoic acid, indicating that exchange of tritium with ring-bound hydrogen occurs under the conditions of this experiment.

#### A MICROHYDROGENATION TECHNIQUE

In the present author's laboratory, an apparatus has been devised for operation on the 1-standard-ml. scale (15). This volume of 97% tritium gas has an activity of nearly 3 curies, and, since hydrogenations can be carried nearly to completion without the use of carrier hydrogen, the method is applicable to the preparation of compounds of very high specific activity. In this respect, therefore, the method appears to differ essentially from those of Williams and Ronzio and of Bernstein and co-workers in whose techniques the use of carrier-free tritium would necessitate the use of impossibly high total activities.

The apparatus is shown in Fig. 71. Tritium is contained in the bulb  $Tr$ , which is opened in the usual way at a break-seal, and the space between stopcocks  $S_{55}$  and  $S_{56}$  is used as a gas pipette.  $Tp$  is a Toepler pump, the tube  $C_T$  having been accurately calibrated by weighing mercury as described for the McLeod gauge on page 38. The reaction vessel is a small tube  $V$ , shaped as shown in the figure and of internal volume about 0.5 ml. The part of the apparatus to which it is attached, namely that part bounded by stopcocks  $S_{58}$ ,  $S_{59}$ , and the surface of the mercury in the bulb  $R$ , is constructed of thick-walled tubing of internal diameter about 2 mm. The total capacity of the whole of this part, including the reaction vessel, is thus no more than about 1 standard ml.

As the maximum head of mercury ( $h$ ) available in the Toepler pump is considerably less than 1 atmosphere, the capacity of the reaction assembly can be temporarily increased by lowering the mercury in the reservoir  $R$ . This permits the introduction of 1 ml.

of gas from the Toepler pump. The mercury in *R* is raised and lowered by means of atmospheric pressure and rough vacuum under the control of the two-way stopcock *S*<sub>60</sub>, the movement of the mercury itself being controlled with stopcock *S*<sub>61</sub>.

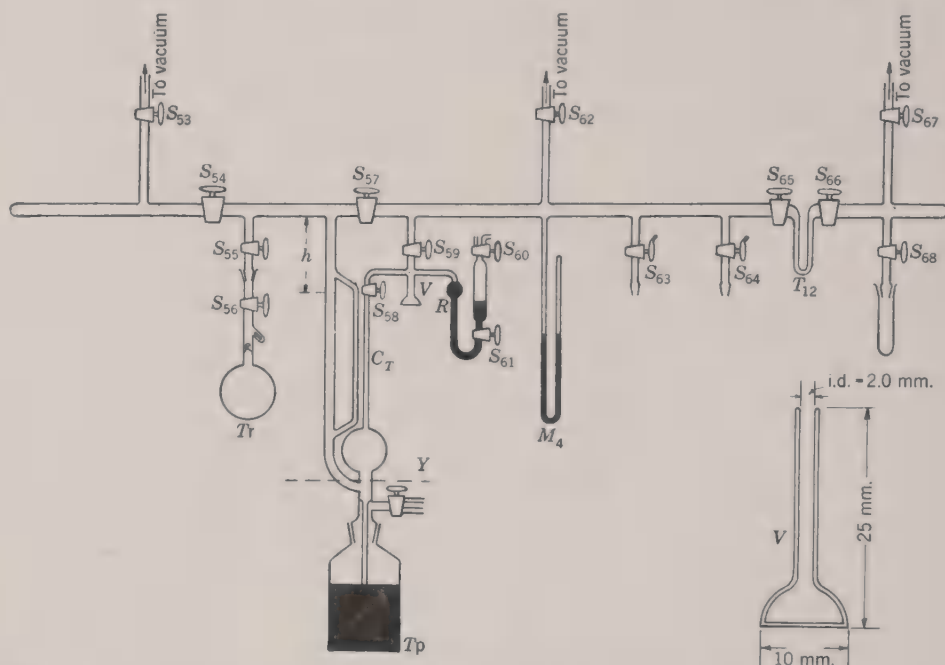


FIG. 71. Permanent vacuum-line apparatus for tritiation of double bonds on micro scale.

*C*<sub>T</sub>: Calibrated tube on Toepler pump.

*h*: Maximum head of mercury attainable for compression of gas past *S*<sub>58</sub>.

*M*<sub>4</sub>: Manometer.

*S*<sub>53</sub>–*S*<sub>68</sub>: Stopcocks.

*T*<sub>12</sub>: U-trap.

*T*<sub>r</sub>: Bulb of tritium.

*T*<sub>p</sub>: Toepler pump.

*R*: Mercury reservoir (for altering capacity of adjoining apparatus).

*V*: Reaction vessel (details inset).

*Y*: See text.

### *Micro-scale Preparation of Tritium-labeled Hexoestrol*

As in the method of Williams and Ronzio, the reaction used here is that of Campbell, Dodds, and Lawson (13). The catalyst used, however, is palladium black instead of palladium supported on charcoal because that catalyst gives rise to oily products containing, presumably, a mixture of *meso*- and *iso*hexoestrol. On the

other hand, palladium black, the catalyst used by Campbell, Dodds, and Lawson in their original work (16), yields solid products consisting predominantly of *mesohexoestrol*.

*Palladium catalyst.* This is prepared according to the method of Heilbron, Sexton, and Spring (17): 1 gm. palladous chloride is digested with 1 liter of water on the steam bath for 1 hour while being stirred continuously. To the hot solution, 2 ml. of 80% formic acid is added followed 5 min. later by 50 ml. of a 10% solution of potassium hydroxide. The solution is kept on the steam bath with continuous stirring for a further two hours and the precipitated palladium metal then filtered off, washed well with hot water, and dried in a desiccator.

*Hydrogenation.* About 6 mg. of dienoestrol recrystallized from ethanol (needles; melting point  $227^{\circ}$ – $228^{\circ}$ ) is accurately weighed into a previously constructed reaction vessel (V in Fig. 71) and 3–4 mg. palladium black added. The tube is then sealed into position on the apparatus and very carefully evacuated. Care is necessary because gas desorbed from the catalyst as the pressure diminishes may cause it to become scattered about the apparatus. About 50  $\mu$ l. sodium-dried dioxane is measured into a small tube which is then attached to the apparatus below stopcock  $S_{63}$ , chilled in liquid air, and evacuated. The dioxane is distilled into the reaction vessel, for which purpose it is convenient to have a miniature Dewar vessel, of about 25 mm. outside diameter, which will fit into the confined space about V. While the transfer is going on an equivalent or a slight excess of tritium is admitted to the Toepler pump, using the space between stopcocks  $S_{55}$  and  $S_{56}$  as a pipette, on the basis of 1 mg. dienoestrol = 167 standard  $\mu$ l. hydrogen. For this measurement it is convenient to have previously ascertained the dead-space constant for the instrument as described for the McLeod gauge (p. 42); i.e., the factor by which the volume of gas trapped in the bulb and measured in the calibrated tube has to be multiplied in order to give the volume of gas in the whole instrument. Then, with the reaction vessel still cooled in liquid air, the mercury in R is lowered to the bottom of the bulb and the tritium in the Toepler pump pumped past stopcock  $S_{58}$  into the reaction assembly. In the



author's apparatus, four strokes of the pump are sufficient to achieve virtually quantitative transfer. The mercury in *R* is then raised to the top of the bulb, thus compressing the gas to about 1 atmosphere pressure. Because the volume of the reaction vessel assembly is so small (about 1 ml.) compared with the volume of the Toepler bulb (about 250 ml.), the volume of gas taken may now be checked by setting the level of mercury in the Toepler pump just above the junction of the bulb with the side tube (i.e., at *Y* in Fig. 71) and opening stopcock *S*<sub>58</sub>; this stopcock is then immediately closed again, the mercury level in the Toepler pump raised in the usual way, and a reading taken. No dead-space correction factor now need be applied, all the gas having been measured. Close agreement with the former reading should be observed. The mercury in the bulb *R* is now lowered, the gas in the pump compressed past stopcock *S*<sub>58</sub>, and the mercury level in *R* raised again. Finally, the liquid air is removed from around the reaction vessel.

The hydrogenation proceeds at laboratory temperature and its progress may be studied by taking measurements of the residual gas at intervals. This is done by cooling the reaction vessel in liquid air for about 10 min. to ensure that all solvent is condensed, withdrawing the gas into the Toepler bulb as already described, and taking a reading. The mercury level in *R* is then lowered, the unreacted gas compressed back into the reaction vessel, the mercury level in *R* raised again, and the liquid air removed.

The reaction proceeds much more slowly than in a larger apparatus of more conventional design. This is partly because on a larger scale it is usual to supply an excess of hydrogen so that the pressure does not diminish sufficiently in the course of the reaction to slow it up, and partly because stirring or shaking is possible on a larger scale, thus exposing a relatively larger liquid surface to the gas. The speed of the reaction in the micro-apparatus is determined by a number of factors, and particularly by the size and shape of the reaction vessel. If the bottom is quite flat and in a horizontal plane, the solution forms a thin film over the bottom with the catalyst more or less evenly distributed; the

reaction then proceeds more quickly than if, because of a concavity or a tilt, the solution forms a drop with a smaller surface and with the catalyst lumped together in a small heap. Owing to the difficulty of making these conditions absolutely identical in different experiments, it has not been possible to confirm the observation of Williams and Ronzio that the reaction proceeds more quickly in the presence of an appreciable activity of tritium. With most experiments carried out in the author's laboratory, however, the reaction is about 95% complete within 24–48 hours. As an example of the method, an actual experiment may be quoted. In this and in that described later for the preparation of labeled stearic acid, the tritium-hydrogen used contained a nominal 0.5% of tritium and should therefore have had a specific activity of about 13 mc./ml. From the results obtained it appeared that the specific activity was, in fact, about half this value. Incorporation of tritium proceeded satisfactorily, however, and there seems no reason to doubt that the reaction could be promoted with tritium of the highest available specific activity, namely 2.7 curies/ml. (97%  $T_2$ ).

First, 4.2 mg. palladium black and 7.7 mg. dienolestrol were weighed into a reaction vessel which was then sealed into position, evacuated, and about 50  $\mu$ l. dioxane distilled into it. The weight of dienolestrol taken is equivalent to 1285 standard  $\mu$ l. hydrogen. The actual volume of tritium-hydrogen measured out was 1420  $\mu$ l., which was pumped into the reaction vessel assembly in four strokes of the Toepler pump. This gas was recovered back into the Toepler bulb, as already described, for a second measurement which constituted the zero time reading for the reaction. The second measurement was 1360 standard  $\mu$ l., which is 4.4% lower than the first. This is a larger error than is usually obtained and cannot be attributed to the gas left behind in the reaction vessel assembly because that accounts for no more than 0.4%.

The tritium was then compressed back into the reaction vessel assembly in the manner described, the Dewar vessel of liquid air removed, and the reaction allowed to proceed at laboratory temperature. Two more readings were taken that day and then

again the following morning. Figure 72 shows that within  $4\frac{1}{2}$  hours the reaction was 72.8% complete and within 25 hours it was 93.2% complete. After 44 hours (96%), when the speed of the reaction had become very slow, the residual tritium ( $130\ \mu\text{l.}$ ) was withdrawn into the Toepler pump, the dioxane distilled into a tube for disposal, and the reaction vessel cut down.

A small quantity of acetone was added to the reaction vessel and, when the product had dissolved, the solution was carefully pipetted off into a small tube and centrifuged to remove catalyst.

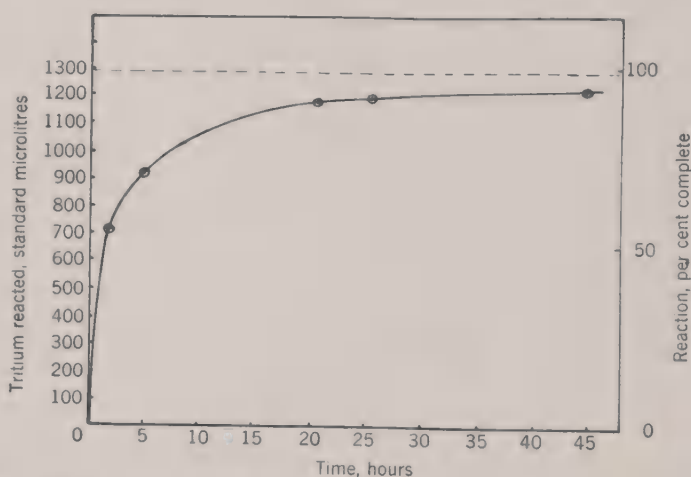


FIG. 72. Curve showing rate of tritiation of dienoestrol in apparatus shown in Fig. 71.

The solution was again pipetted off into another weighed tube and the solvent evaporated off by means of a jet of hydrogen directed at the surface. The product thus recovered weighed 7.7 mg. which is exactly the weight of the dienoestrol originally taken. It was dissolved in 1 ml. *N*/10 sodium hydroxide solution and reprecipitated by the addition of an exact equivalent of standard acid to displace any tritium which may have been introduced into the phenolic groups. The precipitate was centrifuged off, washed, recentrifuged, dried, and recrystallized from benzene-ligroin. On the second recrystallization, 6.1 mg. of material melting at  $176^{\circ}$ – $178^{\circ}$  was obtained. This melting point was only  $7^{\circ}$  lower than that of an authentic specimen of hexoestrol, indicating



that the product was not seriously contaminated with impurities. No attempts at further purification on this scale have yet been made.

*Activity.* After suitable dilution with carrier hexoestrol, wet combustion and assay by the method described on page 193 were carried out. The product was found to contain  $7.0 \times 10^8$  counts/min./mg. From other work the activity of the tritium-hydrogen used was known to be  $5.68 \times 10^9$  counts/ml. and, since 1.23 ml. had been incorporated into 7.7 mg. hexoestrol, its specific activity should have been  $9.1 \times 10^8$  counts/min./mg. It is thought that some of the tritium must therefore have exchanged with the phenolic groups and been subsequently eliminated by the procedure adopted for that purpose.

### *Tritium-labeled Stearic Acid*

By a procedure exactly similar to that described above, labeled stearic acid may be prepared from elaidic acid. Elaidic acid is the *trans* isomer of oleic acid and is used because oleic acid, being liquid, is difficult to purify from other unsaturated acids. Elaidic acid, however, is a solid melting at  $45^\circ$  and can be obtained in a state of high purity by synthetic procedures. Hence, by using it in the tritiation reaction instead of oleic acid, the danger of producing labeled acids other than stearic or of producing stearic acid labeled in other than the 9,10 position is much reduced.

In an actual experiment, 3.9 mg. palladium black and 14.4 mg. crystalline elaidic acid were introduced into a reaction vessel which was then sealed to the apparatus and evacuated. 50  $\mu$ l. dry dioxane was added by distillation *in vacuo* and 1150  $\mu$ l. tritium-hydrogen (theoretical volume required = 1148  $\mu$ l.) was measured out in the Toepler pump and compressed by the standard procedure into the reaction vessel assembly. It was left over the weekend, by the end of which time (46 hours) all but 53  $\mu$ l. gas had been consumed, the reaction therefore being 96% complete. The reaction vessel was cut down, the contents extracted with ether, and the solution centrifuged free from catalyst. On evaporation to dryness, 14.0 mg. solid acid were recovered (97%).



*Assay.* 1.25 mg. product was accurately diluted in two operations, by a factor of  $7.8 \times 10^6$ , with inactive carrier stearic acid. Some of the diluted material was combusted and assayed for tritium as tritio-butane in the usual way. Another portion of it was converted to the calcium salt before combustion and assay. The observed activities were 435 counts/min./mg. for the free acid and 450 counts/min./mg. (calculated to the free acid) for the calcium salt. This close agreement indicates that no tritium had entered the carboxyl group. The activity of the original undiluted material was calculated to be  $3.5 \times 10^8$  counts/min./mg., that is, nearly 0.5 mc./mg. taking into account the efficiency of the assay procedure.

The tritiated hexoestrol and stearic acid prepared by this method afford examples of labeled materials used to solve two different kinds of problem. The hexoestrol is required in very high specific activities so that it may be traced at physiological dose levels (about 0.2  $\mu$ g. in the rat). The stearic acid, on the other hand, is required for work in which the  $C^{14}$ -labeled compound is prohibitively expensive at least for preliminary experiments. For investigations into the contribution of dietary long-chain fatty acids to milk constituents in the goat, high activities (about 5 mc. for each experiment) are required. This is readily obtained at negligible cost if tritium is used. Whereas for the preparation of hexoestrol of maximum possible specific activity the micro-scale method is essential, any of the published methods would serve for the preparation of labeled stearic acid since its specific activity is unimportant. The micro-scale apparatus, however, being applicable to the preparation of both classes of compound, probably has advantages of convenience over the others.

#### REFERENCES

1. E. J. WILSON, A.E.R.E. Memorandum I/M 28. (July, 1953).
2. J. O. HALFORD and L. C. ANDERSON, *J. Am. Chem. Soc.* **58**, 736 (1936).
3. W. E. VAN HEYNINGEN, D. RITTENBERG, and R. SCHOENHEIMER, *J. Biol. Chem.* **125**, 495 (1938).
4. C. K. INGOLD, C. G. RAISIN, and C. L. WILSON, *J. Chem. Soc.* **1936**, 1643.

5. A. I. VOGEL, "A Text-Book of Practical Organic Chemistry," p. 457. Longmans Green, New York, 1948.
6. C. HORREX and M. POLANYI, *Mem. Proc. Manchester Lit. & Phil. Soc.* **80**, 33 (1935-36).
7. K. BLOCH and D. RITTENBERG, *J. Biol. Chem.* **149**, 505 (1943).
8. H. S. ANKER and K. BLOCH, *J. Am. Chem. Soc.* **66**, 1752 (1944).
9. D. K. FUKUSHIMA and T. F. GALLAGHER, *J. Biol. Chem.* **198**, 861 (1952).
10. D. K. FUKUSHIMA and T. F. GALLAGHER, *J. Biol. Chem.* **198**, 871 (1952).
11. J. BELL and S. J. THOMSON, *J. Chem. Soc.* **1952**, 576.
12. D. L. WILLIAMS and A. R. RONZIO, *J. Am. Chem. Soc.* **72**, 5787 (1950).
13. M. R. CAMPBELL, E. C. DODDS, and W. LAWSON, *Proc. Roy. Soc. (London)* **B128**, 253 (1939-40).
14. I. A. BERSTEIN, W. BENNETT, and M. FIELDS, *J. Am. Chem. Soc.* **74**, 5763 (1952).
15. R. F. GLASCOCK, in press.
16. W. LAWSON, private communication.
17. I. M. HEILBRON, W. A. SEXTON, and F. S. SPRING, *J. Chem. Soc.* **1929**, 926.

## CHAPTER XII

### THE STUDY OF *IN VITRO* OXIDATION

When the total amount of labeled substrate carbon oxidized to carbon dioxide during the course of an *in vitro* experiment is required, a useful technique is that of Chernick, Masro, and Chaikoff (1). Their method is designed for solid counting of carbon dioxide in the form of barium carbonate. The apparatus consists of a 50-ml., stoppered, conical flask with side arm and a center well extended through the bottom of the flask. This well contains a rolled up filter paper and is sealed with a rubber cap.

The tissue slices in a suitable medium (usually bicarbonate Ringer), in which is dissolved the labeled substrate, are placed in the flask and 0.2 ml. 2*N* sulphuric acid is placed in the side arm. The air in the flask is displaced by a gas mixture, usually consisting of 95% oxygen and 5% carbon dioxide, and the flask stoppered and shaken in a constant temperature bath for the required time. Five minutes before the end of the experiment, about 1 ml. 30% potassium hydroxide solution is injected with a syringe through the rubber cap on to the filter paper in the center well. Then, at the end of the experiment, the flask is tilted so that the acid runs into the medium, stopping the reaction and displacing carbon dioxide from the solution into the gas phase. The flask is set aside for 30 min. to allow the carbon dioxide in the gas to be absorbed in the alkali on the filter paper which is then removed, and the carbonate extracted from it and precipitated as barium carbonate for solid counting.

#### PERMANENT-VACUUM-LINE GAS COUNTING METHOD

##### *Total Activity Appearing as Carbon Dioxide*

If a gas analysis apparatus is available, an alternative method, used currently in the author's laboratory, may prove more con-

venient than one depending on solid counting. The apparatus consists of a 50-ml. flat-bottom flask with standard joint (B24) to which a head, designed as shown in Fig. 73, is fitted. The tissue slices and medium are placed in the flask and the head, with both stopcocks open, is attached, the ground surfaces being lubricated with high-vacuum grease. The unit is then clamped in the constant-temperature water bath and the air displaced by passing the usual oxygen-carbon dioxide gas mixture in through stopcock *B* and allowing it to run to waste at *J*. When all the air

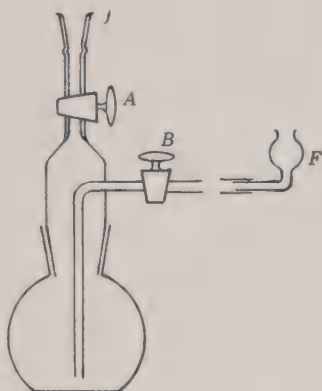


FIG. 73. Incubation unit for *in vitro* oxidation measurement. *A*, *B*: Stopcocks, *F*: Small funnel for acid; *J*: Standard joint.

has been displaced, stopcocks *B* and *A* are closed in that order and the unit incubated with shaking for the usual time.

At the end of the experiment the flask is attached to the high-vacuum apparatus below stopcock  $S_{27}$  as shown in Fig. 74, the additional trap *U*, cooled in a solid carbon dioxide bath, being added to prevent excessive amounts of water entering the apparatus and clogging the traps.  $T_6$  and  $T_7$  are cooled in liquid air as for a combustion and a small funnel of capacity about 1 ml. is attached with rubber tubing to the side arm of the incubation unit. The apparatus is evacuated as far as stopcock *A* and the control stopcock  $S_{25}$  closed. *A* is then opened, thus allowing the gas in the unit to expand into the apparatus. The small funnel on the side arm is filled with 5*N* sulphuric acid and stopcock *B* care-



fully opened to allow the acid to be drawn into the flask. Care is taken not to allow the unit to be flooded with air after the acid has been drawn in.

With the vacuum apparatus open to the pumps, as for a combustion, the control stopcock is cautiously opened and the gas

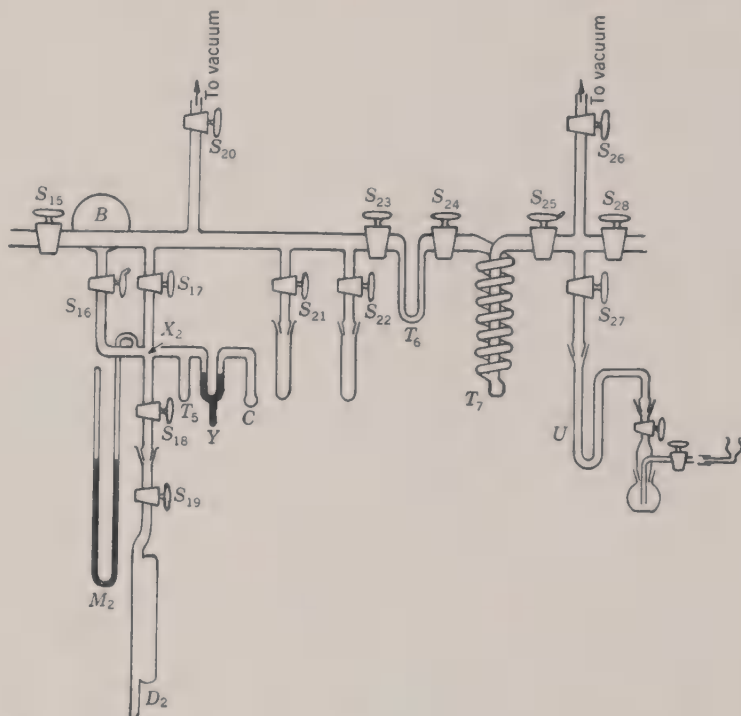


FIG. 74. Permanent vacuum line apparatus for measurement and assay of  $C^{14}O_2$  produced in *in vitro* experiment. (Part of main apparatus shown in Fig. 1.)

*B*: Carbon dioxide reservoir.  
*C*: Carbon disulphide reservoir.  
*D*<sub>2</sub>: Gas counter.  
*S*<sub>15</sub>–*S*<sub>28</sub>: Stopcocks.

*T*<sub>5</sub>, *T*<sub>6</sub>, and *T*<sub>7</sub>: Condensation tube and traps.

*U*: Additional trap (see text).

*X*<sub>2</sub>: Manometric chamber.

*Y*: Mercury cut-off.

mixture in the unit is pumped through the traps. The contents of the flask will ultimately boil and are allowed to do so for about 30 sec. to ensure that all the gas is displaced. The carbon dioxide in the traps *T*<sub>6</sub> and *T*<sub>7</sub> is then separated from any water that may have escaped the trap *U*, by sublimation at  $-78^\circ$  into the man-

ometric chamber where its volume is measured. Its specific activity is determined by the method already described in Chapter IV and the total activity in the whole sample calculated. From a knowledge of the specific activity of the substrate, the total weight of substrate carbon oxidized to carbon dioxide during the period of the experiment can then be calculated.

### *Specific Activity of Respiratory Carbon Dioxide*

It is sometimes useful to be able to measure the *specific activity* of the respiratory carbon dioxide as well as the *total activity* produced during the experiment. The specific activity of the respiratory carbon dioxide is its volume divided into the total activity. The volume of respiratory carbon dioxide is the total volume of carbon dioxide less that added in the buffer system at the beginning of the experiment. This quantity could be found by carrying out a blank experiment on each unit after charging with medium and gas but it is easier to calculate it from a knowledge of (a) the percentage of carbon dioxide in the gas mixture and Ringer, respectively, and (b) the capacity of each incubation unit.

The percentage of carbon dioxide in the gas mixture is ascertained by filling a vessel of known volume (e.g., an incubation unit or a 100-ml. flask fitted with a stopcock) with the mixture, pumping it through the trapping system, and measuring the carbon dioxide obtained (see Table 7). The carbon dioxide content of the Ringer can be found by a direct measurement after addition of acid in the manner described or, if it is made up accurately from pure reagents, by calculation.

The incubation units should be marked so that each flask is always united with the same head and the capacity of each is determined by weighing empty and full of water. If the liquid medium is always accurately pipetted out, and the tissue slices accurately weighed, the gas space above them in the unit will also be accurately known. The only new measurement that need ever be made once all these values have been determined is the carbon dioxide content of the gas when a new cylinder is put into service.

TABLE 7

EXAMPLE OF CALCULATIONS NECESSARY TO DETERMINE ZERO TIME CARBON DIOXIDE CONTENT OF BUFFER SYSTEM USED IN *in vitro* OXIDATION EXPERIMENT

<i>Analysis of cylinder gas</i> (nominally 95% O <sub>2</sub> : 5% CO <sub>2</sub> )		
Capacity of vessel used for sample		145 ml.
Barometer		755 mm.
Temperature		293°K
Therefore corrected volume of gas taken is		
$\frac{145 \times 273 \times 755}{293 \times 760}$	=	134.5 std. ml.
Carbon dioxide extracted (2 experiments)		6.5 std. ml.
Hence, carbon dioxide content of mixture		4.83 %
<i>Calibration of incubation unit No. 8</i>		
(Used in an experiment with 0.5-gm. slices in 5 ml. medium.)		
Capacity of unit		119.5 ml.
Space occupied by medium plus slices		5.5 ml.
Gas space (at 37° C.)		114.0 ml.
Therefore, corrected volume of gas taken (at 760 mm.) is		
$\frac{114 \times 273}{310}$	=	100.3 std. ml.
Saturated water vapor pressure at 37° C.		47 mm.
Barometer		755 mm.
Therefore, pressure of gas in flask is		708 mm.
Therefore, corrected volume is		93.5 std. ml.
Therefore, carbon dioxide content is $93.5 \times \frac{4.83}{100}$	=	4.52 std. ml.
Carbon dioxide content of 5 ml. medium (2 experiments)		2.85 std. ml.
Total carbon dioxide content of flask at start of experiment		7.37 std. ml.

In Table 7 is given an example of the measurements made. The zero-time carbon dioxide of the buffer system in the particular unit referred to works out at 7.37 standard ml. In any experiment carried out in that unit, therefore, the volume of respiratory carbon dioxide is found by deducting this volume from the volume of the total carbon dioxide.

In Table 8 is given an example of the kind of results that can be obtained by this technique. Lactating mammary gland slices from sheep were incubated in bicarbonate Ringer (gas phase: 95% oxygen, 5% carbon dioxide) containing 0.02 molar radio-

TABLE 8

A SELECTION OF RESULTS FROM DIFFERENT EXPERIMENTS SHOWING THE EFFECT OF GLUCOSE (0.3%) ON THE OXIDATION OF LABELED ACETATE (0.02M) BY LACTATING SHEEP MAMMARY GLAND SLICES INCUBATED IN BICARBONATE RINGER (GAS PHASE 95% O<sub>2</sub>, 5% CO<sub>2</sub>)

Experi- ment	I Substrate	II	III	IV	V	VI	VII*
		Volume final CO <sub>2</sub> (std. ml.)	Volume initial CO <sub>2</sub> (std. ml.)	Volume respira- tory CO <sub>2</sub> (std. ml.)	Total activity (counts/ min.)	Sp. act. of resp. CO <sub>2</sub> (c./ min./ ml.)	Rate oxid. C <sup>14</sup> (c./ min./hr.)
30	1-C <sup>14</sup> acetate	6.65	5.26	1.39	1540	1108	9.6
	1-C <sup>14</sup> acetate + glucose	7.70	5.11	2.59	1872	725	10.7
31	1-C <sup>14</sup> acetate	5.11	4.63	0.48	1348	2809	8.0
	1-C <sup>14</sup> acetate + glucose	5.95	4.67	1.28	2184	1707	11.4
32	1-C <sup>14</sup> acetate	6.00	5.26	0.74	2148	2903	10.7
	1-C <sup>14</sup> acetate + glucose	7.50	5.59	1.91	3292	1724	18.0

It is to be noted that, in the presence of glucose, more acetate carbon is oxidized (column VII) than in its absence but that the specific activity of the respiratory carbon dioxide (column VI) is lowered because of dilution by inactive carbon dioxide derived from the glucose. [From Duncombe and Glascock (2).]

\* Corrected to 1 mg. dry weight of tissue.

active sodium acetate as substrate and in the presence and absence of glucose (0.3%). It will be seen that the total activity appearing as carbon dioxide (column V, expressed more accurately in column VII where it is corrected to unit dry weight of the slices and reduced to unit time) is always increased by the presence of glucose. That is to say, glucose stimulates the oxidation of acetate carbon. It will be seen that the effect of glucose is also to *reduce* the specific activity of the respiratory carbon dioxide (column VI). This must be because the glucose itself is oxidized and the inactive carbon dioxide derived from it dilutes the carbon derived from the radioactive acetate. That glucose



itself is oxidized is also suggested by the much greater volume of respiratory carbon dioxide (column IV) obtained when both substrates are present than when acetate alone is present.

These results are presented only as an example of an application of the technique and it is not suggested that in themselves they are of very great moment. They serve to illustrate the fact, however, that the technique may yield results not easily obtained by other methods.

#### REFERENCES

1. S. S. CHERNICK, E. J. MASRO, and I. L. CHAIKOFF, *Proc. Soc. Exptl. Biol. Med.* **73**, 348 (1950).
2. W. G. DUNCOMBE, and R. F. GLASCOCK, *Biochem. J.* **55**, xxiii (1953), and unpublished results.

# APPENDIX

## STANDARD TAPER JOINTS\*

SHOWING APPROXIMATE EQUIVALENCE BETWEEN BRITISH AND AMERICAN STANDARD JOINTS IN MOST COMMONLY USED SERIES. (DIMENSIONS IN MILLIMETERS.)

<i>British</i>				<i>American</i>			
<i>Joint size</i>	<i>Diameter</i>		<i>Min. length</i>	<i>Diameter</i>		<i>Joint size</i>	<i>Min. length</i>
	<i>Large end</i>	<i>Small end</i>		<i>Large end</i>	<i>Small end</i>		
B5	5.0	3.5	15	5.0	3	5/20	20
B7	7.5	5.7	18	7.5	5	7/25	25
B10	10.0	7.8	22	10.0	7	10/30	30
B12	12.5	10.1	24	12.0	9	12/30	30
B14	14.5	12.0	25	14.5	11	14/35	35
B16	16.0	13.4	26				
B19	18.8	16.0	28	18.8	15	19/38	38
B24	24.0	21.0	30	24.0	20	24/40	40
B29	29.2	26.0	32	29.2	25	29/42	42
B34	34.5	31.1	34	34.5	30	34/45	45
B40	40.0	36.4	36	40.0	35	40/50	50
B45	45.0	41.2	38	45.0	40	45/50	50
B50	50.0	46.0	40	50.0	45	50/50	50
B55	55.0	50.8	42	55.0	50	55/50	50
B60	60.0	55.6	44	60.0	55	60/50	50

\* Table kindly supplied by Quickfit and Quartz Ltd.



## AUTHOR INDEX

Numbers in parentheses are reference numbers and are included to assist in locating references when the authors' names are not mentioned in the text. Numbers in italics indicate the page on which the reference is listed at the end of the chapter.

- |  |  |
|--|--|
| <p>Abrams, R., 6, 9<br/>           Allen, M. B., 146, <i>156</i><br/>           Anderson, E. C., 68, 71, 73, 74<br/>           Anderson, L. C., 208, 228<br/>           Anderson, R. C., 11, 32, 47, 106, <i>115</i><br/>           Anker, H. S., 214, 229<br/>           Armstrong, W. D., 87, 97, <i>115</i><br/>           Arnold, J. R., 71, 74<br/>           Arrol, W. J., 53 (3), 71 (20), 72, 73,<br/>               74, 158, <i>179</i></p>   | <p>Calvin, M., 3 (2), 9<br/>           Campbell, I. G., 65, 73, 111, 114,<br/>               <i>115</i>, 159, 171, <i>179</i><br/>           Campbell, M. R., 216, 222, 229<br/>           Chaikoff, I. L., 1 (1), 9, 230, 236<br/>           Chasson, R. L., 190 (3), <i>194</i><br/>           Chernick, S. S., 230, 236<br/>           Clark, L., 6, 9<br/>           Cohn, M., 136, <i>156</i><br/>           Crathorn, A. R., 71, 73, 74</p>  |
| <p>Ballentine, R., 62, 65, 70, 73, 74,<br/>               158, 168, 169, <i>179</i><br/>           Barbour, H. G., 135, <i>156</i><br/>           Barker, H., 71, 74<br/>           Bell, J., 215, 229<br/>           Bennett, W., 164, <i>179</i>, 219, 229<br/>           Bernstein, W., 62, 65, 70, 73, 74,<br/>               158, 168, 169, <i>179</i><br/>           Berry, C. E., 135, <i>156</i><br/>           Berstein, I. A., 164, <i>179</i>, 219, 229<br/>           Biggs, M. W., 146, <i>156</i>, 164, 167,<br/>               <i>179</i><br/>           Bloch, K., 213, 214, 229<br/>           Bothner-By, A. A., 11, 32, 47, 106,<br/>               <i>115</i><br/>           Bradley, J. E. S., 69, 74, 130, <i>133</i><br/>           Brown, S. C., 62, 73, 75, 86<br/>           Brown, W. G., 178, <i>179</i><br/>           Brownell, G. L., 51, 53, 73<br/>           Buchanan, D. L., 21, 30, 47</p> | <p>Damon, P. E., 6 (6), 9<br/>           Dauben, W. G., 1 (1), 9<br/>           Delabarre, Y., 11, 32, 47, 106, <i>115</i><br/>           Dennstedt, M., 112, <i>115</i><br/>           Dodds, E. C., 216, 222, 229<br/>           Dubbs, C. A., 146, 149, <i>156</i><br/>           Duncombe, W. G., 110 (9), <i>115</i>,<br/>               117 (2), 133, 235, 236<br/>           Dunshee, B., 87 (1), <i>115</i><br/>           Du Vigneaud, V., 158 (5), 169 (5),<br/>               170 (5), <i>179</i><br/>           Eidinoff, M. L., 6 (7), 7, 9, 64, 73,<br/>               157, 158 (5), 169 (5), 170<br/>               (5), <i>179</i><br/>           Entenman, C., 1, 9<br/>           Evans, R. D., 70 (14), 74<br/>           Farkas, A., 44 (8), 47<br/>           Fields, M., 164, <i>179</i>, 219, 229</p> |



- Folch, J., 31 (5), 47, 89 (3), 115  
 Freedman, A. J., 68, 73  
 Friedel, R. A., 152, 156, 173, 179  
 Friedman, L., 70 (14), 74, 156  
 Frodyma, M. M., 34, 47, 102, 115  
 Fukushima, D. K., 214, 229  
  
 Gallagher, T. F., 214, 229  
 Glascock, R. F., 5, 9, 11, 47, 71  
     (20), 72, 74, 110 (9), 115,  
     116, 117 (2), 133, 159, 179,  
     182 (2), 194, 221, 229, 235,  
     236  
 Gould, R. G., 157, 179  
 Graff, J., 146, 147, 156  
  
 Halford, J. O., 208, 228  
 Hall, L. G., 135, 156  
 Hamilton, W. F., 135, 156  
 Harfenist, M., 160, 179  
 Harper, W. W., 59, 73, 78, 86  
 Hawkings, R. C., 70, 74  
 Hayes, F. N., 157, 179  
 Heidelberger, C., 3 (2), 9  
 Heilbron, I. M., 223, 229  
 Henriques, F. C., Jr., 56, 73, 146,  
     156, 159, 164, 179  
 Holloway, R. C., 69, 74, 130, 133  
 Holt, P. F., 196, 198, 201  
 Hopson-Hill, B. I., 196, 201  
 Horrex, C., 212, 229  
 Hughes, B. P., 198, 201  
 Hunter, R. F., 70, 74  
  
 Ingold, C. K., 210, 228  
 Irsa, A. P., 156  
  
 Janney, C. D., 164, 179  
  
 Kamen, M. D., 168, 179  
 Kaplan, L., 178, 179  
 Kelsey, F. E., 6 (5), 9  
 Keston, A. S., 135, 143, 156  
  
 Kirk, M. R., 146, 156, 164, 167, 179  
 Kline, L., 160, 179  
 Knoll, J. E., 6 (7), 7, 9, 157, 158  
     (5), 169 (5), 170 (5), 179  
 Kopfer, F., 112, 115  
 Kritchevsky, D., 146, 156, 164, 167,  
     179  
  
 Lawson, W., 216, 222, 223, 229  
 Lerner, S. R., 1 (1), 9  
 Libby, W. F., 71, 74  
 Lindenbaum, A., 97, 115  
 Lockhart, H. S., 51, 53, 73  
  
 McFarlane, M. S., 69, 74, 130, 133  
 MacKnight, M. L., 190 (3), 194  
 Mann, W. B., 70, 74  
 Margnetti, C., 56, 73, 146, 156, 159,  
     164, 179  
 Marshall, R. O., 146, 156  
 Masro, E. J., 230, 236  
 Melville, H. W., 44 (8), 47  
 Miller, W. W., 62, 70, 73, 74, 75, 86  
 Moyer, B. J., 164, 179  
  
 Nakao, A., 21, 30, 47  
 Naughton, J. J., 34, 47, 102, 115  
 Neher, H. V., 59, 73, 78, 86  
 Neill, J. M., 89 (2), 115  
 Niederl, J. B., 103, 115  
 Niederl, V., 103, 115  
 Nier, A. O., 70 (14), 74  
  
 Orchin, M., 152, 156, 173, 179  
  
 Pace, N., 160, 179  
 Parkinson, G. B., 70, 74  
 Payne, P. R., 65, 73, 111, 114, 115,  
     159, 171, 179  
 Pickering, W. H., 78, 86  
 Plazin, J., 68, 73, 89 (3), 115  
 Polanyi, M., 212, 229

- Popják, G., 141, 143, 156  
Powell, T. M., 182, 194
- Rachele, J. R., 158 (5), 169 (5),  
170 (5), 179  
Raisin, C. G., 210, 228  
Reid, A. F., 168, 179  
Reid, E. B., 182, 194  
Reid, J. C., 3 (2), 9  
Reinmann, Arnold L., 44 (7), 47  
Rittenberg, D., 135, 143, 146, 147,  
156, 196, 201, 210, 212, 213,  
214, 228, 229  
Robinson, C. V., 13, 47, 156, 174,  
179  
Ronzio, A. R., 216, 229  
Roth, H., 102, 115  
Ruben, S., 146, 156
- Schachman, H. K., 160, 179  
Schoenheimer, R., 135, 143, 156,  
210, 212, 228  
Schubert, J., 97, 115  
Sexton, W. A., 223, 229  
Sharpe, J., 6 (8), 9  
Singer, L., 87 (1), 115  
Smith, G. N., 146, 156  
Spring, F. S., 223, 229  
Steele, R., 68, 73, 89 (3), 115
- Taylor, D., 6 (8), 9, 51, 73  
Thermal Syndicate, Ltd., 118 (3),  
133  
Thomson, S. J., 215, 229  
Tolbert, B. M., 3 (2), 9, 164, 179  
Troost, A., 60, 73
- Van Heyningen, W. E., 210, 212,  
228  
Van Slyke, D. D., 31, 47, 68, 73, 89,  
115  
Verly, W. G., 158, 169, 170, 179  
Vogel, A. I., 211, 229
- Washburn, H. W., 135, 156  
Weisiger, J. R., 89 (3), 115  
Wender, I., 152, 156, 173, 179  
White, D. F., 65, 73, 111, 114, 115,  
159, 171, 179  
Williams, D. L., 216, 229  
Wilson, C. L., 210, 228  
Wilson, E. J., 53 (3), 73, 158, 179,  
203, 228  
Wilzbach, K. E., 178, 179
- Yankwich, P. E., 3 (2), 9
- Zbarsky, S. H., 87 (1), 115

## SUBJECT INDEX

- Acetic acid, H<sup>\*</sup>-labeled, 129, 208, 215
- Acetophenone, hydrogenation of, 219
- Acetylene, measurement of C<sup>14</sup> as, 71 ff.
- Alpha particles, 48, 52, 58
- Aluminum carbide, 171 ff.
- Amines, contamination of N<sub>2</sub><sup>15</sup> by, 195
- Amino acids, N<sup>15</sup> assay of, 196 ff.
- Ammonia, as an intermediate in N<sup>15</sup> assay, 195 ff.
- $\Delta^4$ -Androstene-3, 17, dione, 215
- Anode resistance, 59
- Anode wire, thickness of, in counters, 62
- Anthracite, 73
- Asbestos, 119, 150 ff.  
    platinized, 122
- Azobenzene, 196
- Backscattering, 2 ff.
- Barium carbonate, radioactive, back-scattering factor of, 3  
    preparation of, 94, 97, 110  
    solid counting of, 3
- Beta-particles, ionization by, 48  
    from C<sup>14</sup>, 1, 52 ff.  
    from tritium, 54
- n*-Butane, 117, 128, 132, 156, 160, 180 ff.  
    assay of tritium as, 180 ff.  
    factors affecting yield of, 189  
    preparation of, from water, 182  
    purity of, 187
- Butane-filled counters, 190
- iso*-Butane, 192
- Butene, 187
- n*-Butyl bromide, 183
- n*-Butyl magnesium bromide, 182 ff.
- sec*-Butyl chloride, 154
- Calcium metal, reduction of water with, 146
- Carbon, heavy (C<sup>13</sup>), 109, 116, 128, 133  
    in deuterio-hydrocarbons, 152, 155  
    radioactive (C<sup>14</sup>), calculation of specific activity, 2  
    gas phase assay of, as acetylene, 71 ff.  
    as carbon dioxide, 48 ff., 75 ff.  
    infinite thickness value, 2  
    maximum energy of beta-particles of, 1  
    solid counting of, 1, 2, 6, 94, 97, 110
- Carbon dioxide, assay of radioactivity of, in Geiger counters, 70, 75 ff., 109, 127  
    in ionization chambers, 53 ff.  
    in proportional counters, 66 ff., 93, 109  
    desorption from charcoal, 129 ff.  
    measurement of, 26 ff., 42, 86, 92, 103, 107, 124, 127  
    purification of from tritium contamination, 129  
    removal of nitrogen oxides from, 102 ff., 109, 130

- reservoir, 29, 85, 127, 188
- respiratory, 233
- Carbon dioxide-carbon disulphide filled counters, 75 ff., 127
- Carbon disulphide, charging reservoir with, 84
- effect on carbon dioxide-filled counters, 76 ff.
- introduction into counters, 82, 127
- manipulation in vacuum system, 47, 70
- Cathode, chromium, 62
- copper screen, 162
- gold, 62
- graphite, 62, 69, 80 ff., 173, 190
- mild steel, 73
- negative ion formation at, 75
- passive iron, 190
- silver, 62, 66, 170
- stainless steel, 128, 190
- Characteristic of counters, 61, 76, 162, 171, 173, 190
- Charcoal trap, 129
- Charge collected at various anode voltages, 49 ff.
- Charge transfer gas, 75 ff.
- Charged molecules, 48
- Cholesterol,  $H^{\bullet}$ -labeled, 214
- Combustion, of deuterio compounds, 107 ff., 136, 138, 147
- dry, 72, 102, 106 ff., 111, 136, 138, 147, 200
- using permanent vacuum line technique, 116 ff.
- isotopic fractionation during, 87
- of labeled compounds, 87 ff., 116 ff., 136 ff., 147, 200
- on large scale for radiocarbon dating, 72
- of tritium-hydrogen, 203 ff.
- wet, 88, 97, 98, 194
- of tritium labeled compounds, 194
- Van Slyke fluid for, 89
- Combustion tube, 103, 106, 112, 118 ff., 147
- Counter circuit, fundamental, 59
- Counters, 58 ff.
- characteristics of, 61, 76, 162, 171, 173, 190
- gas, efficiency of, 83
- Geiger, butane filled, 190 ff.
- Geiger,  $CO_2$ - $CS_2$  filled, 75 ff.
- applications of, 109, 127, 230 ff.
- baking manifold for, 81
- behavior of, 82
- design of, 80
- efficiency of, 83
- filling of, 83 ff., 128
- paralysis of, 81
- putting into service, 80
- general construction of, 62 ff.
- Geiger, 48, 52, 75 ff.
- end window, 1
- gas flow, 1
- self-quenching, 60
- proportional, 49 ff., 63 ff., 93, 109
- acetylene-filled, 71 ff.
- carbon dioxide filled, 68 ff.
- carbon dioxide-methane filled, 66, 93
- hydrogen containing, 168 ff.
- methane filled, 171 ff.
- screen wall, 71
- Counting, gas, of  $C^{14}$ , 48 ff., 75 ff.
- properties of various gases, 159
- of tritium, 157 ff., 180 ff.
- solid, 1, 9, 97
- compared with gas counting, 4
- Current, direct, amplifier, 51 ff.

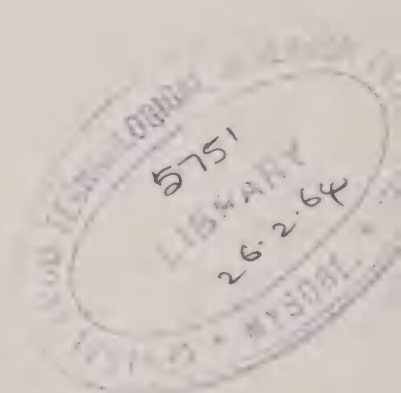


- Currents ion, measurement of, 51 ff.  
stress, 51
- Cut-offs, mercury, 23, 44, 70, 127
- Dating, radiocarbon, 71
- Dehydroisoandrosterone, 215
- Deuterium, determination of, 110, 134 ff.  
by falling drop method, 135 ff.  
in mass spectrometer using hydrocarbons, 152  
using hydrogen, 134, 146  
using water, 135
- Deuterium-hydrogen, ions from, formed in mass spectrometer, 134
- Deuterium-labeled compounds, preparation, of, 202 ff.  
cholesterol, 213  
lauric acid, 213  
palmitic acid, 210
- Deuterium oxide, 146, 210
- Dibutyl ether, 175
- Dienoestrol, 216 ff., 223 ff.
- Discharge, quenching of, in counters, 59  
tubes, elementary theory of, 48
- Doser, gas, 128
- Drift, rate of, 56, 167
- Elaidic acid, 227
- Electrometer, vibrating reed, 51, 53, 56, 164, 167, 178
- Electronic equipment, 61, 66, 68, 70, 78, 171, 173, 191
- Electroscope, Lauritsen, 56, 166
- End-window counters, 1 ff.
- Ethane, preparation for deuterium assay, 153 ff.
- Ether, dry, for Grignard reagent, 183
- Exchange, contamination of equipment by: see Memory effects
- of hydrogen between water and malonic acid, 208
- labeling of fatty acids by, 210 ff.
- Falling drop method, 135 ff.  
apparatus, 141
- Fatty acids, H<sup>3</sup>-labeled, 210 ff.  
triply labeled, 127, 133
- Flowmeter, 122 ff.
- o*-Fluorotoluene, 135, 143 ff.
- Furnace, electric, 106, 113, 117 ff.
- Gas, charge transfer, 75 ff.
- Gas amplification in counting tubes, 50, 52
- Gas counters, 49, 62 ff., 75 ff., 93, 109, 128, 190
- Gas manipulation and measurement in a vacuum, 26 ff.
- Gas phase condensation of carbon dioxide, 21, 121
- Gas phase measurement of radioactivity, 4 (summary)
- Geiger counters, 48, 52, 63 ff., 70, 75 ff., 190 ff.  
threshold, 50, 60
- Glassblowing, 24
- Glass cutting, 25
- Glass, exchangeable hydrogen in, 134  
heat resistant, 178  
Pyrex, 22  
tape, 118 ff., 184
- Glucose, as digestion catalyst, 196  
radioactive, 82
- Grease, vacuum, 17, 132, 193, 203
- Grignard reagents, 156, 174 ff., 182 ff.
- Halogens, removal of from combustion products, 102, 137, 141
- Hexoestrol, 216 ff., 222 ff.
- Hydrazine, 91

- Hydrazobenzene, determination of  $N^{15}$  in, 198
- Hydrogen-deuterium mixtures: ions from in mass spectrometer, 134
- Hydrogen, exchangeable in organic compounds, 208 ff.
- as medium for deuterium assay, 146
- for tritium assay, 163 ff.
- micro-determination of, 102 ff.
- Hydrogenation, catalytic, 216 ff., 221
- of acetophenone, 219
- of dienoestrol, 216, 222
- of elaidic acid, 227
- Hydrobromite, sodium, 196
- Inconel combustion tube, 112
- Incubation unit, 231
- Insulator, in ionization chamber, 51 ff.
- Ion, currents, 50 ff.
- multiplication, 50, 52
- pairs, 48
- peaks from  $N^{15}$ , 195, 200
- Ionization, 48 ff.
- Ionization chambers, 50 ff.
- Ionization chamber methods, of isotopic gas analysis, 53 ff., 164 ff.
- Iron, isotopes, 7
- Isotopes, assay of several in same sample, 7, 116 ff.
- Isotopic fractionation of deuterium-hydrogen, 134
- of  $C^{14}$  during combustion, 87
- of deuterium in deuterio-ethane synthesis, 155
- of tritium in tritio-methane synthesis, 173, 177
- Joints, standard taper, 19, 237
- Laboratory, isotopic gas analysis, design of, 10 ff.
- Lauric acid, 213
- Lead castle for gas counters, 79
- Lithium aluminum hydride, 146, 167
- Lysine, 196
- Magnesium amalgam, 146, 164, 174
- Magnesium perchlorate, anhydrous, regeneration of, 122
- Magnesium turnings, 146, 164, 183
- Malonic acid, tritiated, 208
- Manometers, simple U-tube, 27 ff.
- two-liquid, 32, 103, 108
- Van Slyke type 30, 90 ff.
- Manometric apparatus, Van Slyke, 88
- Manometric chamber, 26, 29, 30, 84, 104, 124, 187, 191
- Manometric chamber calibration of, 29, 104
- Memory effects, 132, 149, 155, 158, 163, 168, 169, 193, 203
- Mercury, effect on proportional counters, 13, 178
- Mercury diffusion pumps, 13
- Methane, as medium for deuterium assay, 152
- for tritium assay, 114, 171 ff.
- Methane, vapor pressure of at liquid nitrogen temperature, 175
- Methane internal gas flow proportional counters, 6
- Methane proportional counters, 66 ff., 93, 169 ff.
- Methane reservoir, 66, 93
- Methanol, 103, 109
- Methyl magnesium iodide, 175
- Micropipette, 143
- Monitoring apparatus, 132, 193
- 2-Naphthol, 1-amino-4-sulphonic acid, 115
- Nickelic oxide, 178

- Nitric acid, removal from combustion water, 137 ff., 141
- Nitrogen, heavy ( $N^{15}$ ), assay of, 195 ff.
- natural abundance, 195
- Nitrogen oxides, effect on counters, 109, 130
- reduction to nitrogen for  $N^{15}$  assay, 195, 200
- removal of, from combustion  $CO_2$ , 102 ff., 109, 130
- Nitrogenous compounds, combustion of, 105 ff., 107, 109, 130 ff.
- Nitrometer, 200
- Oestrone,  $H^*$ -labeled, 215
- Oil for two-liquid manometers, 34
- Oxidation, biological *in vitro*, 230 ff.
- Oxygen supply and purification, 102 ff., 108, 113, 122, 136, 148
- Palladium, 203, 218, 223
- Palmitic acid, 126, 149
- $H^*$ -labeled, 210 ff.
- Plateau, 61 ff., 76 ff., 162, 171, 173, 190
- Platinum, 103, 106, 112, 122, 206
- Platinum oxide catalyst, Adams, 211, 215, 219
- Progesterone,  $H^*$ -labeled, 215
- Proportional counters, 48, 63 ff., 68 ff., 169 ff. (See also Counters, gas)
- Proportionality, region of limited, 50
- Quenching, agents, 60
- electronic, 59, 70, 78
- resistance, 59
- self, 60
- Radioactive carbon, see Carbon
- hydrogen, see Tritium
- Radiolysis, of tritiated water, 206
- Sample-to-background ratio, 68, 73
- Sample size for solid and gas counting, 4
- Scaffolding, 14 ff., 22
- Seals, tungsten-to-glass, 62, 81
- Self-absorption, 1 ff.
- Silicone, fluid, 82, 144, 191
- grease, 184
- Solid counting, of  $C^{14}$ , 1 ff., 6
- of tritium, 7, 157
- Spectrometer, mass, 129, 134, 146, 187, 195
- Steroids,  $H^*$ -labeled preparation of, 213 ff.
- Stopcocks, 15 ff.
- "control," 19, 113
- grease, contamination, by tritium, 132, 193, 203
- Styrene,  $H^*$ -labeled, 219
- Testosterone,  $H^*$ -labeled, 215
- Thermocouple, 119, 131
- Thermostat, 142
- Thickness, infinite, 2, 157
- Timing of falling drop, 145
- Toepler pumps, 26, 43, 148, 155, 166, 198, 207, 221, 225
- Traps, 13, 19 ff., 120 ff.
- Tritium, 52 ff., 111 ff., 147, 157 ff., 180 ff., 202 ff.
- availability and properties, 157, 225
- contamination of combustion  $CO_2$  by, 129
- determination of, 157 ff., 180 ff.
- choice of medium, 158
- as butane, 180 ff.
- as hydrogen, 163 ff.
- as methane, 171, 178
- as hydrogen-methane, 178
- as water, 160
- diffusion through palladium, 203
- half-life, 157

- infinite thickness of, 2, 157
- precautions in handling, 202
- survey of counting methods, 158
- Tritium-labeled compounds, 202 ff.
  - acetic acid, 208
  - fatty acids, 210 ff.
  - hexoestrol, 216 ff., 221 ff.
  - malonic acid, 208 ff.
  - methyl phenyl carbinol, 220
  - stearic acid, 227 ff.
  - steroids, 213 ff.
  - styrene, 219 ff.
  - sulphuric acid, 202
  - water, 203 ff.
- precautions in combusting, 132
- wet combustion of, 133, 193
- Vacuum, gas manipulation in, 26
  - rough, 23
- Vacuum apparatus, general requirements and layout, 10 ff.
- Van Slyke combustion fluid, 89, 97, 100, 133, 194
- Voltage and charge collected in discharge tube, 48 ff.
- Water, as medium for mass spectrometric assay of deuterium, 135
  - tritium counting, 160 ff.
- Water, conversion to butane, 182 ff.
  - to ethane, 153
  - to methane, 171, 175, 178
- purification in falling drop method, 137 ff., 140
- reduction to hydrogen, 147 ff.
- Water, tritiated, preparation from tritium-hydrogen, 203 ff.
- Water bath for falling drop apparatus, 141
- Water vapor, measurement of, 32, 103, 109, 160 ff.
  - elimination of in McLeod gauge measurements, 42
- Xanthydrol, C<sup>14</sup>-ureide, 87









01/11/89

C. F. T. R. I. LIBRARY, MYSORE.

Acc. No.

5751

Call No.

GX3,BI N54

✓ MC 17.6.80

30/9/87

Please return this publication on or before the last DUE DATE stamped below to avoid incurring overdue charges.

P. No.

Due Date

Return date

CHECKED  
2008

VERIFIED  
2013

To be issued from 11/5/69  
P. S. K. Mayimdar

CFTRI-MYSORE



5751

Isotopic gas ana.

G X3, BI N54

COCK

c Gas

rio-



